NASA/CP—2001–(in press)

Mars Sample Handling Protocol Workshop Series

Interim Report of the Workshop Series
Workshop 2a (Sterilization) Final Report
Arlington, Virginia
November 28-30, 2000

Edited by:

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June 2001

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PREFACE

The Space Studies Board of the National Research Council provided a series of recommendations to NASA on planetary protection requirements for future Mars sample return missions. One of the Board's key findings suggested that, although current evidence of the martian surface suggests that life as we know it would not tolerate the planet's harsh environment, there remain "plausible scenarios for extant microbial life on Mars."[1] Based on this conclusion, all samples returned from Mars should be considered potentially hazardous until it has been demonstrated that they are not.

In response to the National Research Council's findings and recommendations, NASA has undertaken a series of workshops to address issues regarding NASA's proposed sample return missions. Work was previously undertaken at the Mars Sample Handling and Protocol Workshop 1 (March 2000) to formulate recommendations on effective methods for life detection and/or biohazard testing on returned samples.

The NASA Planetary Protection Officer convened the Mars Sample Sterilization Workshop, the third in the Mars Sample Handling Protocol Workshop Series, on November 28-30, 2000 at the Holiday Inn Rosslyn Westpark, Arlington, Virginia. Because of the short timeframe between this Workshop and the second Workshop in the Series, which was convened in October 2000 in Bethesda, Maryland, they were developed in parallel, so the Sterilization Workshop and its report have therefore been designated as "2a"). The focus of Workshop 2a was to make recommendations for effective sterilization procedures for all phases of Mars sample return missions, and to answer the question of whether we can sterilize samples in such a way that the geological characteristics of the samples are not significantly altered.

1 *Mars Sample Return Issues and Recommendations* (1997), Space Studies Board, National Research Council, National Academy Press, Washington, D.C., p. 2.

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EXECUTIVE SUMMARY

In 1997, the Space Studies Board of the National Research Council concluded that, while the possibility is remote, there is a chance that life may exist on Mars. As a result, the Board recommended that all samples returned from Mars be considered hazardous until demonstrated otherwise. Because of uncertainties about the evolution and resistance mechanisms of putative martian life, NASA must exercise discretion in planning sample sterilization protocols. In anticipation of a sample return mission from Mars, NASA's Planetary Protection Officer has undertaken a series of workshops to address sample-handling issues. The overall objective of these workshops is to make recommendations for effective sterilization procedures for all phases of Mars sample return missions.

This document is the report resulting from the third workshop in the series, which was held in Arlington, Virginia on November 28-30, 2000. Because of the short timeframe between this Workshop and the second Workshop in the Series, which was convened in October 2000 in Bethesda, Maryland, they were developed in parallel, so the Sterilization Workshop and its report have therefore been designated as "2a"). This report summarizes relevant background information presented at the Workshop, records findings from individual sub-group deliberations, and provides a basis for future workshops on related topics. Specific recommendations and conclusions are not the objective of this report.

At the beginning of Workshop 2a, seven speakers gave plenary presentations. The titles of these presentations were: "Introduction to Mars and Task Assignments," "Microbial Resistance and Biological Control," "Effects of Ionizing Radiation on Macromolecules," "Quantitative Effects of Ionizing Radiation on Proteins and Viruses," "Detection of Microorganisms," "Brief Overview of Sterilization Methods," and "Planetary Quarantine, Spacecraft Sterilization, and Sample Return."

During the Workshop, a number of sub-groups met to deliberate specific, assigned topics. The views and findings expressed by the sub-groups are preliminary in nature and do not necessarily represent a consensus of all workshop participants. Some sub-groups have provided preliminary recommendations for sterilization parameters; these recommendations are intended as a basis for further discussions. A summary of the individual sub-group reports is presented below.

Sub-group 1A: Can survival mechanisms of terrestrial extremophiles serve as models of how putative martian extremophiles might exhibit resistance to sterilization?

Sub-group 1A agreed that terrestrial extremophiles could be used to validate sterilization methods used on Mars samples. However, terrestrial evolution may not present the full spectrum of selective pressures on Mars. The Sub-group recognized that species' adaptations are built by selection, and therefore, life on Mars will be able to survive extremely hostile conditions. While no environment on Earth exactly models that of Mars, there are terrestrial environments that could allow for the isolation of species that are resistant to one or more of the conditions found on Mars. These organisms could be used as models for developing sterilization procedures.

Sub-group 1B: Given (a) the range of uncertainties regarding putative martian life's origins, evolution, and adaptation to extreme environments, (b) our limited knowledge of the biological evolutionary potential on only one planet, and (c) terrestrial biohazard mechanisms of action, what is the worst-case scenario for sterilizing martian samples?

Sub-group 1B agreed that the worst-case scenario would be to find an infectious agent in a returned martian sample. In this case, the sample would need to be kept under containment until an effective sterilization method could be demonstrated. During their deliberations, the participants discussed: 1) approaches to sample handling, 2) the minimum sterilization level needed for the distribution of samples prior to comprehensive life detection, and 3) other fundamental problems for sterilization and risk assessment of putative unconventional martian life forms.

The Sub-group then formulated a set of recommendations based on the assumption that any life form found on Mars would be organic carbon based.

1. All martian material should be brought to a Planetary Protection Level PPL-α (BSL-4 plus) facility where the material would undergo initial testing for life, toxicity, infectivity, and biohazard.
2. Subsequently, sub-samples of the material could then be transferred to a PPL-β (BSL-4) facility or facilities for more extensive evaluation, including tests using live cells and animals.
3. If testing completed in 1 and 2 is negative, samples could be transferred to a BSL-3 facility for additional life detection and environmental hazard testing, followed by transfer to a BSL-2 facility where geological and other tests could be performed under conventional restricted-access laboratory protocols.

Sub-group 1B included supplemental information about scanning X-ray microscopy, an emerging method of life detection (Appendix D).

Sub-group 1C: What sterilization methods and procedures will best preserve the integrity of martian soil and rock samples for future scientific analyses outside the proposed containment facility?

Sub-group 1C reported four preliminary points to be applied to their assessment of various sterilization methods. These were:

1. The martian sample will be returned without exposure to temperatures over 50°C.
2. Sterilization is defined as 10^{-6}.
3. The term decontamination is defined as "make the sample safe to handle."
4. A need for additional information on extremophiles and the effects of conventional sterilization procedures on them.

In addition, the Sub-group specified that an ideal sterilization method would 1) kill and inactivate all living, replicating forms, 2) have no effect on the fundamental properties of the samples, and 3) leave no residual. Using these definitions and constraints as a starting point, the Sub-group then discussed the following sterilization methods: moist heat, dry heat, gamma radiation, ethylene oxide, and low temperature sterilization methods. Each method was assessed in terms of efficacy, temperature and time requirements, penetration, residue, and the physical/chemical/biological impact on the sample. No individual method was preferentially recommended by the participants. However, a combination

of methods, particularly one exploiting the synergistic effect of heat and radiation, should be considered.

Sub-group 1D: If life detection initiatives do not yield evidence of carbon or polymers, is sterilization of representative martian soil and rock samples necessary prior to distribution to awaiting scientist outside the containment facility?

Sub-group 1D revised the question to read "If state-of-the-art life detection efforts do not yield evidence of reduced or organic carbon or regular polymers, are biohazard remediation steps of representative martian soil and rock samples necessary prior to their distribution to waiting scientist outside the containment facility?" This revision was based on the assumptions that the presence of carbon does not necessarily indicate a biosignature, but that carbon-carbon or carbon-hydrogen bonds are presumed necessary for life. The participants then further defined "life detection initiative" to represent a thorough search, using state-of-the-art methods.

The Sub-group subsequently drafted the recommendation that, in the absence of components recognized as essential for life, and the absence of any sign of hazard after a systematic bioassay of a portion of the sample, then no sterilization is required prior to distribution. In submitting this conclusion the participants acknowledged that: 1) there is a possibility that evidence of life might be missed in a fractional sample, 2) that a biohazard must necessarily be comprised of reduced carbon and biopolymers, and 3) investigators who were concerned about potential biohazard could request that samples be sterilized prior to distribution.

The Sub-group also recognized that the issue of sample sterilization is really an issue of tradeoffs based on the scientific requirements being asked of an individual sample. A matrix analysis could be used to assess the tradeoff of biohazard remediation requirements versus the experimental value of the sample. Subsequently, a game theoretical approach to risk analysis was proposed, based on specific biological and chemical rules and other constraints, to predict the risk posed by martian organisms.

Sub-groups 2A and 2B: Given (a) the uncertainties about putative extraterrestrial life in extreme extraterrestrial environments and (b) our limited knowledge about the evolution of sterilization resistance mechanisms in terrestrial extremophiles, can martian samples be sterilized effectively and safely distributed outside the containment facility before life detection, biohazard testing, and other chemical analyses are actually completed and evaluated?

(Sub-groups 2A and 2B met separately but each group addressed the same question.)

Sub-group 2A:

The Sub-group considered many wide-ranging problems concerning sample distribution prior to the completion of hazard testing. These considerations included:

1. Some organisms are slow to show pathogenicity, so a complete analysis of infectivity could take a long time.
2. Because of uncertainty regarding potential hazards, samples must be kept in strict containment, with the possibility of indefinite containment not ruled out.
3. NIH and FDA regulatory guidelines may be useful in developing sterilization procedures.

The Sub-group made four recommendations regarding containment approaches and sterilization methodologies:

1. The sample return facility should have the largest possible containment area.
2. A small (1%) amount of sample could be sterilized and distributed prior to the completion of biohazard evaluation.
3. More research is needed for sterilization procedures on soil and rock.
4. A regulatory framework is needed to guide the development of required protocols.

Sub-group 2B:

Sub-group 2B first specified a number of assumptions to be considered in formulating recommendations for sample distribution protocols. These assumptions include a risk assessment of potential martian life forms, consideration of the current knowledge regarding sterilization effectiveness, and the role that Earth samples should take in modeling sample return protocols. Using the assumptions as a basis, the group then suggested a protocol for determining a sample distribution schedule prior to the completion of all biohazard testing.

A tiered approach to sterilization was recommended; as data regarding the potential hazard of the samples accrue (i.e., life detection tests are negative), sterilization procedures could be reduced in severity. The Sub-group constructed a proposed flowchart depicting three levels of testing to provide a reasonable certainty that distributed samples could be handled safely. The levels of testing are:

Set A: Rapid tests to detect complex organic molecules and organisms infectious to humans.

Set B: A wide variety of tests for infectivity, including *in vivo* tests.

Set C: All tests to detect any type of reasonably possible biohazard.

Sterilization protocols associated with Set A should be severe enough to eliminate even small viruses and hazardous proteins; protocols associated with Set B should eliminate any bacteria and typical viruses. It was acknowledged by the participants that these initial sterilization protocols may render samples useless for some applications.

Lastly, the group recommended that the process of testing and sterilization be thoroughly validated using Earth samples to determine assay sensitivity, sampling statistics, method reproducibility, and operator expertise prior to use on an actual sample.

Sub-group 2C: Can subsamples of preserved martian meteorites serve as models ultimately to test sterilization methods, procedures, and effectiveness?

Sub-group 2C agreed that samples of selected martian meteorites may be used to test sterilization procedures, but only after the procedures had been fully developed on terrestrial analogs. The participants cited two advantages to using actual martian material for testing: 1) the sterilization procedures would be tested as realistically as possible, and 2) the tests would demonstrate the effects of proposed sterilization procedures on the mineralogy and petrography of actual martian rocks. The group recommended that testing should not be confined to biological tests only, but should also include measurements to assess any deleterious effects of the sterilization procedures on the samples.

Sub-groups 3A and 3B: Given the uncertainties, what are effective sterilization methods for samples returned from Mars?

(Sub-groups 3A and 3B met separately but each group addressed the same question.)

Sub-group 3A:

Using assumptions presented by Sub-group 2B, Sub-group 3A formulated several constraints and assumptions in order to develop a sterilization protocol. These were:

1. Assessment of sterilization techniques can be based only upon our existing knowledge of Earth organisms.
2. Initial sterilization doses should be based on an overkill approach.
3. Protocols must be minimally destructive to the sample.

The Sub-group then reviewed a number of known sterilization techniques including ethylene oxide gas sterilization, radiation, dry heat, and a combination of radiation and dry heat. The Sub-group noted the synergistic effect of using a combination of methods, further mentioning that a penetrating method would be more effective than a surface method. The participants then reviewed the sample distribution recommendations from Sub-group 2B, proposing more specific sterilization parameters associated with each test set. These parameters include radiation/dry heat sterilization using a virus model based upon European Pharmacopoeia methods for Test Set A, and lower radiation doses using radiation resistant organisms as models for Test Set B. Sample distribution without sterilization was recommended for Test Set C.

Sub-group 3B:

Sub-group 3B concluded that the best sterilization approach may be a combination of gamma ray or high-energy electron exposure plus simultaneous dry heat. This conclusion is based upon the assumption that chemical elements on Mars are the same as on Earth and therefore sterilization conditions for Earth microorganisms should eradicate microorganisms of similar size from Mars.

The participants initially looked at the effect of radiation on geochemistry, concluding that gamma radiation in specific doses does not induce radioactivity or produce measurable changes in rock and mineral isotopic/elemental crystallographic structure. It was concluded that exposure to gamma rays or high-energy electrons provides a feasible sterilization option, further defining the sterilization procedure itself, suggesting that the procedure for sterilization consist of gamma irradiation at a temperature of approximately 105°C.

Irradiation conditions were examined based on the assumption that martian organisms are similar to terrestrial organisms. Because martian organisms could be more resistant to radiation, the group recommended that it may be prudent to explore hypothetical possibilities for the evolution of martian organisms adapted to high radiation environments. Current evidence indicates that a radiation dose of 55 Mrads will kill all known infectious agents on Earth. Thus, the Sub-group noted that 400 Mrads would give a large safety margin, even under worst-case scenarios.

Finally, Sub-group 3B highlighted nine experiments to provide more information to develop sterilization protocols. These are:

1. Study microorganisms growing in high radiation environments.
2. Collect and obtain new data on extremophiles and traditional microbes to obtain estimates of required heat/radiation doses.
3. Determine the most difficult organisms to sterilize by these methods.
4. Carry out sterilization experiments on simulated martian samples, followed by martian meteorite material.
5. Study bond energies of non-carbon polymers and limits on nucleic acid repair.
6. Examine current hazard containment methods.
7. Test all aspects of sterilization methods on simulated martian rock.
8. Compare sterilized and unsterilized martian meteorite material.
9. Determine upper limits on bacterial populations that can exist on rock.

Sub-group 3B's report includes supplemental reports on "Sterilization by Ionizing Radiation" and "Life Forms Based on Silicon Polymers."

Notes

This document is the final report of Workshop 2a, but only an interim report of the Workshop series. This report is intended to provide a summary of Workshop 2a to serve as background information for participants of future workshops in the series and any other interested parties. *If any portion of this document is to be cited or referenced it must be with the understanding that this document is neither authoritative nor indicative of any final decisions or plans for future Mars missions.*

This Executive Summary was drafted from summaries written by each sub-group following Workshop 2a. The complete summaries, which appear in the main body of this report, have undergone minimal editing. No attempt has been made to reconcile differences between the sub-groups, nor to determine at this time whether particular suggestions would be feasible or recommended for a Mars sample return mission. The information herein will eventually be integrated with additional findings and recommendations from the entire Workshop series. Upon completion of the Workshop series, a final report for the series will be published.

INTRODUCTION

Terrestrial extremophiles survive some of the harshest environments on Earth. For example, the bacterium *Deinococcus radiodurans* has evolved unique genetic repair mechanisms that make it resistant to a range of high radiation doses. Even pathogenic prions exhibit protein conformational changes widely reported to be resistant to excessive heat sterilization. Do these and other unique survival mechanisms have scientific and regulatory implications for NASA in its planning and implementation of sterilization methods and procedures for soil and rock samples returned from Mars?

While terrestrial examples of biological extremophiles like *Deinococcus radiodurans* exist, our knowledge is limited about the underlying genetic repair mechanisms that augment resistance to high radiation doses. The chemical environment of bacteria is known, however, to provide a range of resistance to radiation sterilization, except in those organisms that have evolved unique genetic repair mechanisms. Moreover, the chemical environment of terrestrial microbes may augment adaptations to environmental perturbations that ultimately result in extremophiles. Oncology studies show that eukaryotes exhibit a similar variable chemical resistance to a range of radiation dosages, although our knowledge of the underlying mechanisms also remains limited.

NASA must take into account the uncertainties regarding the biological evolution of resistance mechanisms in terrestrial life when planning biological control measures for potential unique survival mechanisms of putative martian life. How would putative martian microbes evolve, adapt, and survive in the extreme martian environment, even if harsh arid, frigid, and high-radiation exposures on Mars were only intermittent? In light of the scientific uncertainties regarding the evolution of resistance mechanisms in terrestrial extremophiles, NASA must exercise prudence in planning effective sterilization procedures for samples returned from Mars. Moreover, NASA must evaluate which sterilization procedure (amid chemical, gas, heat, and radiation methods) is the most applicable for providing effective biological control in martian soil and rock samples. The foregoing uncertainties surrounding the prospect of sterilizing extraterrestrial soil and rocks is, furthermore, expected to require future research before definitive methods and assurances of sterilization effectiveness develop. Accordingly, the NASA Mars Sample Sterilization Workshop must evaluate the following issues:

1. May survival mechanisms of terrestrial extremophiles serve as models of how putative martian extremophiles might exhibit resistance to sterilization?
2. Given our limited knowledge regarding (a) the evolutionary potential of life on only one planet, (b) putative martian life's origins, evolution, and adaptation to extreme environments, and (c) terrestrial biohazard mechanisms of action, what is the worst-case scenario for sterilizing martian samples?
3. Given (a) the uncertainties of putative extraterrestrial life in extreme environments and (b) our limited knowledge about the evolution of sterilization resistance mechanisms in terrestrial extremophiles, can martian samples be sterilized effectively and then safely distributed outside the containment facility, before life detection, biohazard testing, and other chemical analyses are actually completed and evaluated?
4. What sterilization methods and procedures will best preserve the integrity of martian soil and rock samples for future scientific analyses outside the proposed containment facility?

5. Can subsamples of preserved martian meteorites serve as models ultimately to test sterilization methods, procedures, and effectiveness?
6. If life detection initiatives do not yield evidence of carbon or polymers, is sterilization of representative martian soil and rock samples necessary prior to distribution to awaiting scientists outside the containment facility?
7. Given the uncertainties, what are effective sterilization methods for samples returned from Mars?
8. How should NASA validate and provide warranted assurances of effective sterilization for martian samples?

PLENARY PRESENTATION OVERVIEWS

The workshop began with a series of seven plenary presentations so that all participants could begin with a common basis of background information from which to formulate the discussions in the workshop. Visual aids shown in these talks are presented in Appendix C, including additional information provided by participants.

1. Introduction to Mars and task assignments: John Rummel

Our current understanding of Mars comes from a number of exploratory missions, both orbiting and landing on the planet. The first extensive views of Mars were provided by the Mariner 9 Orbiter, which showed a martian surface with an active geologic history. Scientists noted two seemingly contradictory features: craters, suggesting a dead planet, but with channels and flow features indicative of liquid water. The Viking landing missions included life detection experiments and provided soil chemistry information showing that martian soil is significantly different from that on Earth. NASA's Magellan and Pathfinder missions have provided additional information including evidence of surface frost, volcanic topography, and surface feature data, including a striking flow feature that cuts across surface craters. The presence of flow features suggests that liquid water may have been present on the martian surface during the past 10 million years.

Shortly after the Viking missions touched down on the surface of Mars, scientists discovered oceanic hydrothermal vents on Earth. With that discovery came the realization that bacteria, known as extremophiles, can thrive in environments previously thought to be lethal. Other extremophiles are now known, and particular species are able to withstand extreme conditions, such as dryness, high temperatures, low temperatures, or radiation.

Additional Mars missions are planned over the next decade. The Mars Odyssey orbiter will be launched in the spring of 2001, followed by two landed missions containing rovers in 2003. The first sample return mission is slated for launch as early as 2011, with the current design concept specifying the return of a 1-kilogram sample to the Earth unsterilized.

2. Microbial resistance and biological control: Richard Setlow

The visual materials from this seminar are presented in Appendix C1.

When one looks at the martian surface, the environment of Mars is quite different than that found on Earth. In contrast to Earth, the martian atmosphere is negligible, the magnetic field is negligible, and the surface temperatures are very low (mean surface temperature at the equator is 215 K).

The surface of Mars is bombarded by two major types of radiation: cosmic rays and ultraviolet (UV) light. These radiation sources are not blocked by an atmospheric layer as they are on Earth. Cosmic rays have high kinetic energy and cannot be shielded well. While the estimated dose of cosmic radiation during the round trip is high enough to kill unshielded humans, it is not high enough to kill bacteria. UV radiation also reaches the surface of Mars in doses that are lethal to terrestrial bacteria. It is now known that some organisms can be unusually resistant to UV radiation, particularly at very low temperatures. In addition, the organism's size and cellular structure is paramount to the lethality

of UV. For example, spores and small viruses are more resistant to UV. It is also known that the amount of radiation necessary to kill or inactivate organisms is negatively correlated with size.

Based on the radiation doses available on martian surfaces (18×10^4 J/m^2/24hours), one can infer that bacteria, as we know it, would be killed. However, bacteria under the martian soil surface would be shielded from these radiation sources. Thus, any life present on Mars is likely to dwell beneath the surface.

3. Effects of ionizing radiation on macromolecules: Ellis Kempner

The visual materials from this seminar are presented in Appendix C2.

Ionizing radiation are quanta or particles with considerable kinetic energy. As they pass through matter, they interact and lose some of that energy. In the case of gamma rays and high energy electrons, these interactions occur randomly, principally with orbital electrons. The more electrons, the greater the chance of an interaction.

Most of the effects of ionizing radiation on macromolecules in the liquid water are indirect: radiation products of water (OH– and H+) diffuse to and react with macromolecules. Depending on the particular reaction site, such effects may be damaging to biological activity. More than 99.9% of the radiation damage to macromolecules in aqueous liquid is due to this mechanism. Very little of the damage is due to interactions of the radiations directly on the macromolecules. In the frozen state, water molecules are still the principal species of radiation-damaged molecules, and the same initial species of products are formed. But in this state, they are not free to diffuse. They slowly react with nearby molecules, which are other water molecules (in a cell there are more than 10,000 water molecules for every protein molecule). When frozen or when lyophilized, essentially all of the radiation damage is due to interactions of the radiations directly with the macromolecules. And because of the random nature of gamma rays and high-energy electron ionizations, the larger macromolecules are more likely to be hit. This primary ionization may occur anywhere in the macromolecule. Energy is transferred from the quantum or particle, on average about 60 electron volts (=1500 kcal/mol). Typically, the orbital electron is ejected, but with fairly little kinetic energy; the bulk of the energy is absorbed by the parent molecule as excitations and ionizations. In the case of proteins, these ionizations can occur anywhere in the polypeptide no matter where the original primary ionization occurred. Each hit polypeptide suffers at least one covalent bond breakage in the polymer backbone and also loses all biological activity. One primary ionization in an oligomeric protein leaves one (and sometimes more) subunit with a cleaved backbone. In spite of this damage, the subunits do not dissociate, and the fragments do not disperse.

The radiation damage to carbohydrates differs. In oligosaccharides and glycoproteins, radiation damage is fairly localized to the sugar ring that suffered the initial radiation interaction. No damage appears elsewhere in the molecule. RNA, with sugar rings along the polynucleotide chain, behaves similarly. A primary ionization anywhere in a ribozymal molecule breaks the polynucleotide backbone, but biological activity is not lost unless the primary ionization was directly in the active site. It is anticipated, but not yet proven, that DNA would behave in a similar fashion to radiation.

4. Quantitative effects of ionizing radiation on proteins and viruses: John Battista

The visual materials from this seminar are presented in Appendix C3.

We know that various organisms react differently to radiation doses. One can determine the average dose (or D_{37}) needed to inactivate a live or infectious particle of any kind. For example, the D_{37} dose is about 0.8 Gray for human cells, 30 Gray for E. coli bacteria, and 4000 Gray for tobacco mosaic virus. Spores exclude water and are therefore much more resistance to radiation, with D_{37} values in the thousands of Grays. In developing sterilization procedures involving radiation, NASA will need to take into account bacteria that exhibit radiation resistance. *Deinococcus radiodurans* is a bacterial species that is very resistant to radiation, with a D_{37} value of about 6500 Gray.

It is unknown what selective mechanisms make these bacteria more radiation resistant. As there is no direct selective pressure for this trait, it must be an incidental characteristic. Mutants of *D. radiodurans* that are radiation sensitive are also sensitive to desiccation, so these two traits appear to be functionally correlated. It is known that radiation resistant bacteria have very effective and efficient DNA repair mechanisms when subjected to radiation. But these DNA repair mechanisms are not enough to completely account for their resistance to radiation, and it is believed that there may be other unknown mechanisms that aid in their resistance.

5. The detection of microorganisms: Ronald Schell

The visual materials from this seminar are presented in Appendix C4.

While the study of clinical microbiology may provide some scientific guidance to researchers studying martian samples, it will be important to look more broadly at the diversity of organisms in a variety of environments, and the challenges inherent to detecting these organisms.

In clinical microbiology, 1-20 micron size organisms are considered to be clinically most relevant, with gram-negative bacteria the most detrimental in a clinical hospital setting. Hospitals often examine samples with large numbers of bacteria. For example, one human stool has a bacterial population of 4 to 6 times the human population of the Earth. In addition, many known bacteria (for example, the etiologic agent responsible for leprosy) still cannot be cultured in a laboratory setting. Some researchers estimate that scientists can only culture about 1% of Earth's variety of bacteria. These types of issues may be limiting factors in the detection of martian bacteria. It is also conceivable that a martian bacterial strain could affect humans in a physiological way, but not be *culturable* in the laboratory.

Another significant concern is in the application of current clinical analytical techniques to martian samples with very low concentrations of bacteria. For example, current technologies such as epiflorescent microscopy use various stains to color bacteria or bacterial components for identification and population counts in samples. The stained sample is analyzed microscopically using multiple visual fields and grids to make quantitative determinations. In order to detect bacterial concentrations of less than 10,000/field, sample enrichment techniques must be used. Also, many things, including particles and crystals, can mimic bacteria in a visual field. In a martian sample where a scientist might be looking for one organism, or even a fragment of an organism, in a large surface, current clinical techniques may be lacking.

6. Brief overview of sterilization methods: Carl Bruch

The visual materials from this seminar are presented in Appendix C4.

In the medical field, sterilizing agents can be divided into two categories: chemical agents such as liquids and gases (for example: formaldehyde, ethylene oxide) and physical agents (for example: heat and radiation). Dry heat sterilization at 108-113ºC has been used in previous space missions, such as the Viking Landers. The terminal sterilization of medical products is based on the kinetics of the first order reaction of the sterilant employed. This definition of "sterilization" in this context is "the process by which anticipated levels of microbial contaminants in a load of material are exponentially decreased" by use of D-value calculations "so that the probability of a survivor is 10^{-6} or less." The older concept of sterility testing of sterilized items has limited utility for space missions.

Sterilization for medical applications has two requirements; 1) biological research to assess the kinetics (i.e. D-value) for the sterilant and materials to be sterilized to derive desired doses, and 2) scientific judgement to put all of the necessary parameters (variables) into a physical format that can measure that the desired dosages are achieved in the products to be sterilized. Microbiologists examine various kill cycles (dosages) to determine the D-value to derive a probability of a 10^{-6} sterility assurance level (SAL). The older approach of tests for sterility are non-comprehensive, meaning that one can never test for all possible microbial agents or the conditions necessary for their recovery. Sterility is usually assessed using a single-species biological indicator (BI) or by physical means that employ dosimetric (parametric) release of product based on necessary dosages having been achieved.

One example of medical product sterilization is the use of ethylene oxide gas to sterilize heat-labile materials. The assessment of sterility is usually based on an overkill method that uses a BI challenge. Known amounts of the BI can be seeded into the load to be sterilized. Alternatively, the natural flora, the actual bioburden on the items to be sterilized, can be used as the BI. Fractional cycles of the sterilization dose are run to establish the kill rate (kinetics) for the ETO gas and items to be sterilized; the BIs are retrieved to establish the D-value and are used to extrapolate the probability curve to obtain the 10^{-6} SAL.

Ionizing radiation sterilization of medical supplies is done dosimetrically (i.e., neither BIs nor sterility tests are used). Natural bioburden on product is used in pilot scale tests to establish microbial resistance levels (D-values). The usual method requires 100 samples to be biologically assayed to determine the dose for the 10^{-2} SAL (the verification dose). Newer methods can use as few as 10 samples (dose for 10^{-1} SAL) to establish the D-value. In a mixed microbial population (bioburden), each microbial species has its own radiation D-value, so that a mixed population survivor curve tends to be curvilinear. The most resistant fraction of the bioburden yields the best D-value to establish the 10^{-6} SAL.

There is some synergistic killing effect achieved by simultaneous exposure to low-level dry heat sterilization (below 125ºC) and ionizing radiation. For spores of *Bacillus subtilis*, simultaneous dry heat exposure at 95ºC reduces the ionizing radiation D-value by more than 50%.

7. Planetary quarantine, spacecraft sterilization, and sample return: Martin Favero

The visual materials from this seminar are presented in Appendix C5.

In early planetary missions, such as the Viking mission, the "make and bake" philosophy was the sterilization method of choice to achieve a sterility assurance level of 10^{-6}. The Viking landers, encapsulated in a bioshield, were subsequently dry heat sterilized at 112ºC for a duration of approximately 40 hours. This dry heat cycle was based upon the thermal inactivation characteristics of *Bacillus subtilis* var. *niger* spores with the assumption being that this organism would be representative of other types of spores found on the spacecraft.

After the Viking mission, dry heat sterilization and thermal inactivation experiments were conducted using sterile Teflon ribbons. The basis of these experiments was to look at large number of naturally-occurring airborne spores and to determine experimentally the thermal resistance of the bacterial population. The Teflon ribbons were exposed to the assembly environment for known lengths of time. The ribbons were rolled, inserted into sterile jars, and processed to determine spore populations or heat-treated to determine inactivation times and temperatures. At a test temperature of 125ºC, 8 hours was the longest heating time to yield survivors. Data also showed that wide ranges of D-values were extrapolated, as expected with a non-homogeneous bacterial population.

The Manned Apollo missions were the first to bring back extraterrestrial samples as well as to have quarantine and decontamination issues related to human space travel. The rock boxes containing the Apollo 11 lunar samples were decontaminated with sodium hypochlorite; also dry heat or ethylene oxide were used to "sterilize" the boxes when they were removed from the glove box containment system. The deck that the astronauts walked on after splash down was literally sprayed with a mild germicide (Betadine) as they proceeded from the open retrieval raft in the Indian Ocean. They were then transferred to a quarantine facility. In hindsight these procedures were imperfect and significantly more care will be needed for Mars sample return missions.

SUB-GROUP REPORTS

During the Workshop, the participants were divided into sub-groups to discuss particular issues associated with sample handling, sterilization, and distribution. Each sub-group was given a particular issue or question to deliberate. Guided by a chairperson, each group discussed the issue, developed recommendations, and reported back to the complete Workshop group.

Sub-group 1A

Issue
Can survival mechanisms of terrestrial extremophiles serve as models of how putative martian extremophiles might exhibit resistance to sterilization?

The members of the Sub-group were:
John A. Battista (Chair)
Michael J. Daly
James G. Ferry
Robert Rohwer
Peter Sheridan
David Stahl
Jonathan D. Trent

Recognizing that a species' adaptation to physiological stress evolves through natural selection, it is expected that extant life on Mars will be able to survive extremely hostile conditions. Surface temperatures at the equator of Mars range from –100ºC during the martian winter to 20ºC during the martian summer. Mars is extremely dry; the partial vapor pressure of water on the surface is approximately 0.1 Bars. The martian atmosphere is 95% CO_2 and provides no protection against exposure to 200-300 nm ultraviolet light, which generates strong oxidants from the limited amounts of oxygen present in the atmosphere. It is believed that all organic compounds on the surface of Mars are subject to oxidation by this UV-induced photochemistry. Since this combination of conditions cannot be found anywhere on Earth, it is unlikely that a single terrestrial species will be found that can serve as a surrogate for a putative martian organism when evaluating methods for sterilizing martian samples. There are terrestrial environments, however, that are sufficiently similar to the martian environment to allow the isolation of species that exhibit extreme resistance to a subset of the conditions (e.g., desiccation radiation, or cold) to be encountered on Mars. It is suggested that an effort be made to identify and characterize terrestrial species from environments as similar as possible to those on Mars, and that these species be used to validate sterilization protocols.

Assuming that life on Mars is based on the same building blocks as terrestrial life, only two methods of sterilization were considered viable options, dry heat and gamma radiation, either alone or in combination. These methods will penetrate the sample and, therefore, provide the highest level of assurance that putative organisms will be destroyed. It is recognized that the application of heat will modify the geological properties of the sample. Within reason, every effort should be made to develop and implement a method of sterilization that protects the scientific integrity of the sample.

Consensus

Terrestrial extremophiles can be used to validate methods of sterilization used on Mars samples. However, it is recognized that terrestrial evolution may not present the full spectrum of selective pressures available to martian organisms.

Sub-group 1B

Issue

Given (a) the range of uncertainties regarding putative martian life's origins, evolution, and adaptation to extreme environments, (b) our limited knowledge of the biological evolutionary potential on only one planet, and (c) terrestrial biohazard mechanisms of action, what is the worst-case scenario for sterilizing martian samples?

The members of the Sub-group were:

Indra K. Vasil (Chair)
David W. Beaty
William N. Fishbein
Dean W. Gabriel
Jacques Grange
Ellis Kempner
Richard Knudsen

Discussion

The participants agreed that the worst case scenario would be the finding of an infectious agent in a martian sample. If a biohazard assay were to reveal a toxic effect, all samples would probably need to be kept under BSL-4 containment indefinitely, or until an effective sterilization procedure is demonstrated.

The discussion in this group also ranged across many topics, including the following:

- Conservative approaches were considered. Simply do not bring any samples from Mars to Earth. One participant suggested bringing samples to the moon or the space station for analysis before allowing them to be brought to Earth.
- What is the minimum sterilization needed for distribution of samples prior to the results of hazard testing and life detection? Some suggested that, given the lack of clear indicators of what is being sterilized, the level of sterilization should be set at the maximum level that would be acceptable to the geochemists who would want to study the samples.
- The group considered other fundamental problems. There is a need to consider unconventional (martian) life forms. There will be no way to demonstrate that sterilization has been effective, if no life forms or transmissible toxins are found. The unknowns will make any kind of specific risk assessment difficult.

Recommendations

The following recommendations are based on the assumption that any life found on Mars is organic carbon-based. It is recommended that all of the martian material be brought to a sample receiving facility as soon as possible after the return of the mission where it will be kept at Planetary Protection Level-α (PPL-α = BSL-4 plus). During a period of about 90 days, this material should undergo thorough testing to look for any evidence of life based on determination of organic carbon polymers. This analysis would involve a battery of tests, including mass spectrometry analyses. The material should also be tested for

toxicity, infectivity, and biohazard. These tests would include, but not be limited to, the use of human and other cell lines and would be used in selecting additional tests. In addition, one of the primary purposes of PPL-α is protection of the entire sample from terrestrial contamination.

Sub-samples of material in the PPL-β (BSL-4) facility or facilities will undergo exhaustive testing for toxicity, life detection, infectious nature, and environmental hazards. Initially, NASA will have to consider a number of life detection and biohazard testing methods, some of which were discussed briefly by the participants. Ultimately, the biohazard and life detection tests that NASA selects will include a variety of cell culture and live animal assays.

NASA should also follow technical developments in an emerging life detection method, scanning X-ray microscopy, which presently appears to be an example of another capability that may be applied to examine soil and rock samples returned from Mars. Information about this emerging X-ray microscopy beam-line method was discussed, and is presented here (Appendix D) in a review from the European Synchrotron Radiation Facility (ESRF) in France.

In the event that tests in the BSL-4 facility provide no evidence of organic carbon-based life, and in addition, provide no evidence of a transmissible toxin to humans, animals and plants, the samples may be transferred to PPL-γ (BSL-3) facilities for additional life detection and environmental hazard tests. If evidence of a life form or a transmissible toxin is found, the samples will not be distributed until the nature of the life form or hazard is characterized, or an effective sterilization procedure is demonstrated.

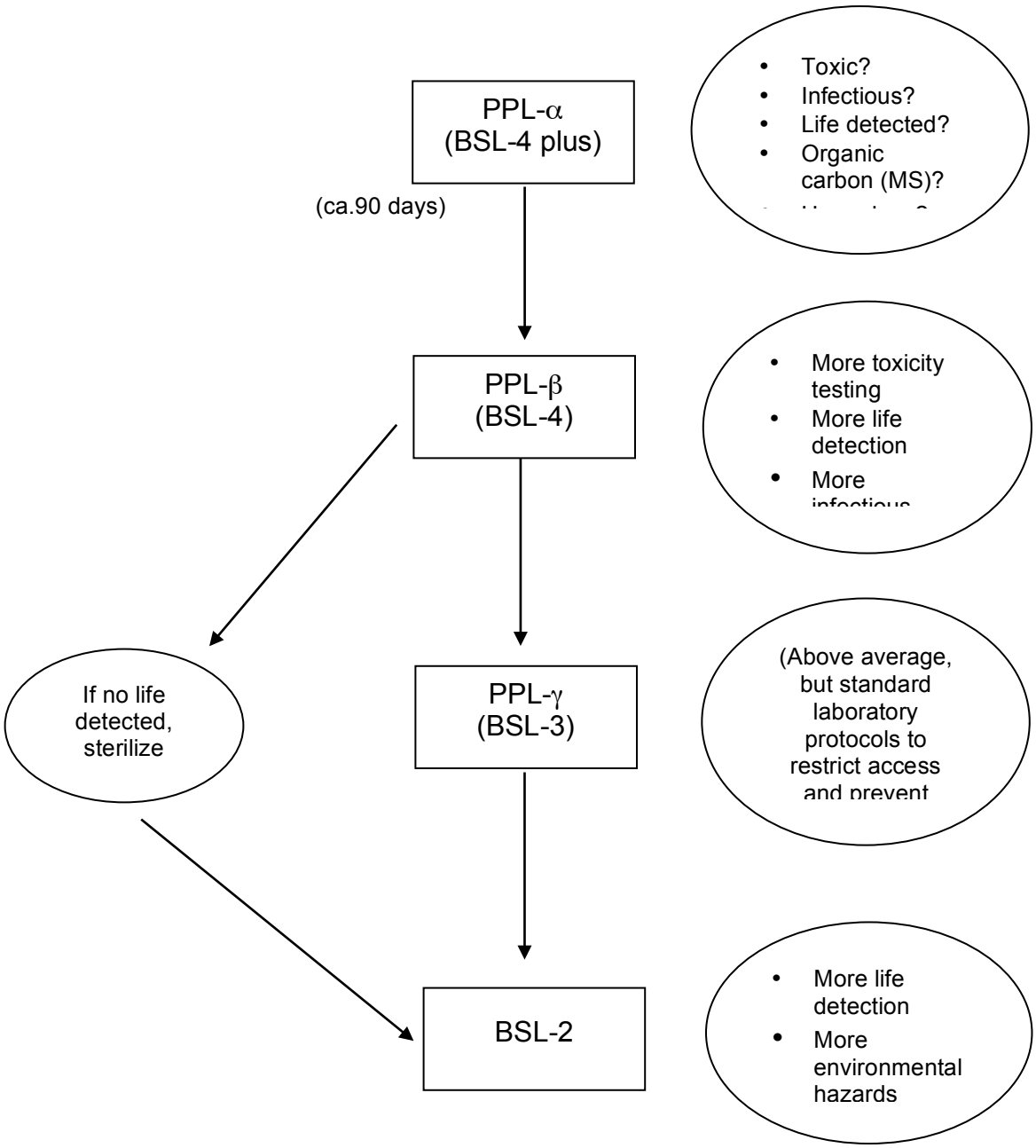
If the additional life detection and environmental hazard testing at the BSL-3 level are negative, the samples will be transferred to a BSL-2 facility with above average but standard laboratory protocols to restrict access and environmental release.

In the event that no evidence of life is found during initial testing in the PPL-β (BSL-4) facilities, some of the samples might be sterilized and transferred directly to BSL-2 facilities for geological and other tests, provided that a convincing protocol for successful sterilization is developed.

Schematic Diagram

The schematic diagram below (see page 18), representing a progression from maximum containment to BSL-2 based on biohazard and life detection considerations, was discussed and adopted by both Sub-groups 1B and 2A.

Progression from
Maximum Containment to BSL-2
Based on Biohazard and Life Detection Considerations
(Adopted by both Sub-groups 1B and 2A)



Sub-group 1C

Issue
What sterilization methods and procedures will best preserve the integrity of martian soil and rock samples for future scientific analyses outside the proposed containment facility?

The members of the Sub-group were:
Martin S. Favero (Chair)
Carlton C. Allen
Carl W. Bruch
Virginia C. Chamberlain
Heinrich D. Holland
Gerhard Kminek
Dimitri Papanastassiou
Irving Pflug
Robert R. Reich
Richard B. Setlow
Pericles Stabekis
Robert W. Walker
John Williams

Preliminary Points
Sub-group 1C considered the following to be important assumptions and preliminary points in guiding their discussion. First, the sample returned from Mars is expected to be exposed to temperatures not exceeding 50ºC during its return to Earth. Second, "sterilization" is defined as 10^{-6} reduction in viable organisms. It is pointed out that this is more extensive requirement than the current standard allowance for spacecraft sterilization (i.e., 10^{-4} per mission). Third, the term "decontamination" is defined as all procedures necessary to make the sample safe to handle. This concept would include the maximum containment approach to sample handling.

Some members of the group suggested that there is a need for information on microorganisms on Earth from extreme environments and studies to determine the effectiveness of conventional sterilization procedures on these organisms.

The ideal sterilization method would:
The ideal method would kill all living, replicating forms in the samples. It would have no effect on the geophysical or geochemical properties of the sample. It would leave no residual. It would have the support of the geological, biomedical, and public health communities, and comply with all regulatory agencies. We need to strive to find the sterilization method or methods that come as close as possible to these ideals.

Methods Discussed:

- **Moist Heat**
  Broad spectrum of efficacy
  121-135ºC for 15 - 40 minutes
  Penetrating for temperature but not moisture
  Would hydrate inorganic and organic components of the sample
  Limited penetration of sample
  Affect and destroy organic and biochemical components
  Easily tailored to Mars Sample Return Laboratory (MSRL) setting

- **Dry Heat**
  Broad spectrum of efficacy
  115 (?)-125-135ºC
  100ºC for 24 hours no changes
  105ºC for 1 month used as an extreme example
  150ºC might start to cause changes; 125ºC less changes
  Penetrating for temperature
  No residual
  May damage sample components depending on temperature and time of exposure.
  Easily tailored to MSRL setting

- **Gamma Radiation**
  Effective against microorganisms
  Some viruses and vegetative bacteria need higher doses
  Penetrating in the context of Mars samples (i.e., 500-1000 g)
  Effectiveness is dose-dependant
  Gamma radiation could be tailored to a MSRL setting
  No sterilant residuals
  Some physical, chemical and biological changes may occur
  Easily tailored to MSRL setting
  Electron beam sterilization not considered because of size and lack of penetration

- **Ethylene Oxide**
  Low temperature
  Broad spectrum of efficacy
  Moisture requirement
  Although penetrating gas it would not penetrate samples
  Residual problem
  Would affect organics by adding ethyl groups
  Explosive and environmental issues
  Easily tailored to MSRL setting

- **Low Temperature Methods**
  Hydrogen peroxide gas plasma sterilization
  Chlorine Dioxide Gas sterilization
  Hydrogen peroxide vapor sterilization
  Broad spectrum of efficacy
  Penetration problems - for surfaces
  Residual may be problem with ethylene oxide; less with oxidizing methods
  Easily tailored to MSRL setting

- **Combination Methods**
  Combinations of methods might also be considered. For example there is a synergistic effect of dry heat and radiation – thermoradiation.

Sub-group 1D

Issue

If life detection initiatives do not yield evidence of carbon or polymers, is sterilization of representative martian soil and rock samples necessary prior to distribution to awaiting scientists outside the containment facility?

The members of the Sub-group were:
Steven A. Benner (Chair)
Daniel Branton
Paul Brown
Harry A. Crissman
Joseph B. Lambert
Frances S. Ligler
Ronald F. Schell
Eric J. Stanbridge

Refinement of the issue
The following observations were first made about the question itself:

(a) Carbon is, of course, an element, and not necessarily a biosignature. Unfortunately, even in its inorganic form (as carbonate in a limestone or a dolomite, for example), carbon can be present as a consequence of life. However, "organic carbon," or "reduced carbon," meaning samples of matter containing carbon-carbon bonds or carbon-hydrogen bonds, are presumed to be necessary for life (at least, carbon-based life).

(b) "Polymer," in this field, is somewhat loosely used to include molecules as diverse as meteoritic kerogens (which are almost certainly not biosignatures, even though they contain reduced carbon) and DNA (which is a regular polymer built from a small number of building blocks.

(c) By "sterilization" the Sub-group inferred "biohazard remediation efforts."

(d) The Sub-group inferred "life detection initiatives" to mean more than a cursory inspection, but rather something that represented "state of the art" tools for detecting biosignatures, at levels in the parts per billion range. Examples of such "life detection initiatives" are found in the Interim Report of Workshop 1 *[Race and Rummel 2000, p. 66].*

Following this discussion, the question was revised to read:

> "If state-of-the-art life detection efforts do not yield evidence of "reduced" or "organic" carbon or regular polymers, are biohazard remediation steps of representative martian soil and rock samples necessary prior to their distribution to waiting scientists outside the containment facility?"

Brief answer to the issue
Briefly, the answer proposed was "No". The Sub-group drafted the following paragraph as recommendation:

> "In the absence of molecular components currently recognized as essential for life (i.e., organic polymers), where such thinking is based on our understanding of terrean life, and given the absence of any sign of hazard after a systematic bioassay of a portion of the sample, then no sterilization is required before distributing the sample to scientists outside the containment facility."

Additional statements
The Sub-group then made three additional statements:

First, the Sub-group recognized the possibility that evidence of life might be missed by sampling, that is, it might be absent in the fraction sampled before sample distribution, but present in the fraction of the sample distributed. The Sub-group noted, however, that a bioassay of the entire sample would be self-defeating, as it would consume the sample.

Second, the Sub-group recognized that its comments were based on the assumption that a biohazard must necessarily be comprised of reduced carbon and biopolymers. The Sub-group did not dismiss out of hand the possibility of non-organic (i.e., non-carbon forms of life, such as replicating clays), although some members of the Sub-group were prepared to do so. But the Sub-group did not see how non-carbon based forms of life would represent a hazard (as an infectious agent) to terrean life. (A successful "parasite" would need to have molecular biology closely analogous to that of their hosts, as these are most likely to best exploit the biochemicals available from the host.) Also, the group could not see how such a biohazard could be remediated, short of dissolution or other total destruction of the sample. Here, "parasite" is defined as an independently evolving form of life that requires a host to survive, and does not contribute a corresponding value to the host (as a symbiont must, by definition). Biohazards can be parasites.

Third, the Sub-group noted that investigators concerned about potential biohazards in the sample could ask to have it sterilized before it is shipped to them.

Additional issues

Ultimately, the issue of sample sterilization is an issue of trade-offs between three factors:

(a) What scientific questions will be asked?
(b) What experiments will be run to answer them?
(c) How will particular methods of biohazard remediation/minimization compromise those experiments?

Analysis of this trade-off would be based on a "matrix table" showing the questions/experiments on one axis, potential remediation/minimization tools on the other, and filled with "yes" and "no" answers to the question: Does the index remediation/minimization tool compromise the ability of the index experiment to answer the scientific question? Information that might be used to construct this matrix table is found in pieces throughout the Interim Report of Workshop 1 *[Race and Rummel, 2000, p. 26].* But without such a matrix table, the discussion of biohazard remediation in returned martian samples can easily become unproductive.

A game theory approach to risk analysis

A "game theory" approach might be applied to the analysis of the sample sterilization problem. In this approach, we try to identify (from our knowledge of life on Earth) certain "universal rules" about life generally. Some of these might be:

Biological rules

(a) The biochemistry of the best parasites is closely similar to that of their hosts.
(b) Living systems develop a hierarchy of use of available resources, in a food chain.
(c) In biological systems, specialists generally beat generalists.
(d) Living systems survive well in their natural environment.

Chemical rules

(a) The 20 amino acids used by life on Earth are not unique. Extraterrestrial life may use others *[Short et al., 1999].*
(b) The 4 nucleobases used by life on Earth are not unique *[Piccirilli et al., 1990].*
(c) A solvent is required for life.

In the "game," we then take the universe of possibilities, and constrain them: for example, we might begin by assuming that the solvent is water. This then suggests further chemical constraints. For example, if the solvent is water, then we know that the natural environment of the organism is rich in nucleophilic reactivity, and operates in a pH range from 0 to 14, which represent the pKas (dissociation constants) of the conjugate acid and conjugate base in water (hydronium and hydroxide,

respectively). Given the biological principle that living systems survive well in their natural environment, organisms evolving in that environment must be relatively insensitive to nucleophiles. This implies that electrophilic reactivity is scarce in the environment.

One consequence of this discussion is the speculation that electrophilic reactivity (e.g., non-nucleophilic reactivity) is the place to search for sterilants. Thus, as terrean life lives in water, it is not surprising that no chemical sterilants for terrean life act as nucleophiles, while many chemical sterilants are electrophiles (e.g., ethylene oxide, glutaraldehyde, formaldehyde). This leads to a conjecture. Ethylene oxide should be a universal sterilant for life forms that have evolved in water as a solvent.

We can, of course, relax the constraint further. For example, we might consider supercritical methane-ammonia as the solvent. Organisms living here must survive in a more nucleophilic environment. Even weaker electrophiles would presumably kill them.

The game theoretic approach can be used for risk assessment. For example, it is quite clear that meteor traffic between Mars and Earth has been frequent. Let us assume that this traffic does not represent a biohazard. The question then becomes, what must be true for NASA sample return to represent a biohazard?

One way that NASA sample return might be hazardous, while natural sample transmission is not, would be for a biohazard to be present in the rocks that NASA returns, but not in the rocks ejected from the surface of Mars via natural processes. Both processes sample Mars non-randomly, of course. NASA will bring samples from the near-surface, and may include sedimentary rocks. Natural sampling prefers igneous rocks, as these are the most likely to maintain their structural integrity during the impact that ejects them. We must then turn to what is known about life on Earth to assess the likelihood that a biohazard present on Mars would be present in the first type of sample, but not the second.

Sub-group 2A

Issue

Given (a) the uncertainties about putative extraterrestrial life in extreme extraterrestrial environments and (b) our limited knowledge about the evolution of sterilization resistance mechanisms in terrestrial extremophiles, can martian samples be sterilized effectively and safely distributed outside the containment facility before life detection, biohazard testing, and other chemical analyses are actually completed and evaluated?

The members of the Sub-group were:

Ronald F. Schell (Chair)
John Battista
Paul Brown
Carl W. Bruch
Robert Rohwer
Virginia C. Chamberlain
Harry A. Crissman
Michael Daly
William N. Fishbein
Jacques Grange

Joseph B. Lambert
Irving Pflug
Robert R. Reich
David Stahl
Indra K. Vasil

Points raised during discussion

This group considered many wide-ranging problems having to do with the distribution of samples before the completion of all life detection, biohazard testing, and chemical analyses:

- Some organisms require more than 90 days to show any pathogenicity. Therefore, a complete analysis of infectious capability could take a very long time to complete in a thorough manner.

- Initially, samples must be kept in strict containment (see schematic diagram, p. 18). This containment must protect from the release of martian material and protect the martian samples from contamination with terrestrial microbes.

- One alternative might be to keep the samples in containment indefinitely. This would require that all experiments be carried out under containment. However, this would make some experiments – thin sections, for example – virtually impossible.

- By analogy, the NIH and FDA have extensive sets of regulatory guidelines. These kinds of guidelines may be useful in developing sterilization procedures that allow the distribution of material from containment.

Recommendations

- It would be desirable for the Sample Return Facility to include the largest possible dedicated containment facility.

- Initially, it may be worth allowing a small amount of the sample (maybe 1%) to be very stringently sterilized, then distributed. However, if this harsh sterilization would make the sample useless, then it would not be worthwhile.

- There is a need for more research on sterilization procedures, particularly as they apply to samples of soil and rock.

- There is a need for a regulatory framework to guide the development of protocols for sterilization.

Schematic Diagram

A schematic diagram, representing a progression from maximum containment to BSL-2 based on biohazard and life detection considerations, was adopted by Sub-groups 1B and 2A. This diagram is presented in the report for Sub-group 1B (see p. 18).

Sub-group 2B

Issue

Given (a) the uncertainties about putative extraterrestrial life in extreme extraterrestrial environments and (b) our limited knowledge about the evolution of sterilization resistance mechanisms in terrestrial extremophiles, can martian samples be sterilized effectively and safely distributed outside the

containment facility before life detection, biohazard testing, and other chemical analyses are actually completed and evaluated?
The members of the Sub-group were:

Frances S. Ligler (Chair)
Daniel Branton
Martin S. Favero
James G. Ferry
Dean W. Gabriel
Ellis Kempner
Richard B. Setlow
Peter Sheridan
Eric J. Stanbridge
Jonathan D. Trent
John Williams

Discussion

Sub-group 2B suggested a protocol for determining when samples could be distributed prior to completion of exhaustive testing for biohazards (see protocol diagram, p. 26). Three levels of testing were established to correspond with different levels of sterilization required to provide a reasonable certainty that distributed materials could be handled safely in terms of a biohazard threat. Test Set A would include rapid tests designed to detect complex organic molecules and organisms infectious to humans. Test Set B would include tests for infection to a wider variety of human cells, plants, and other animals and longer term *in vivo* tests. These tests would require an amount of time intermediate between the rapid Set A and Set C. Test Set C would include all tests important to detect any type of biohazard whose existence is reasonably possible.

As far as which biohazards are reasonably possible, the Sub-group assumed that any life from Mars capable of replication would be carbon based and susceptible to sterilization protocols effective on terrestrial organisms. Organisms growing on Mars are unlikely to colonize terrestrial hosts, and martian viruses are not likely to exist in the absence of martian hosts. However, sterilization protocols should be able to eliminate viruses and hazardous protein in Protocol A and any bacteria and most viruses in Protocol B. The sterilization of any replicating life form (positive result from Test Set C or any prior set of tests) would be developed and validated based on whatever protocol was most effective in destroying the martian life form.

Based on the requirements of scientists planning to study the rocks, the number of levels could be limited to 2 or expanded to 4. For example, if the protocol used to sterilize samples passing Test Set A rendered them useless both for all types of geochemical studies and for external biohazard studies using equipment unavailable in the NASA BSL-4 facility, then there would be no point to distributing the samples after sterilization Protocol A. Sterilization Protocol A will probably include both heat and gamma irradiation. The rationale for sterilization Protocol A is that even though the rapid test battery A showed no evidence of large organic molecules or organisms infectious to humans, the portion of the specimen not selected for testing could harbor biohazards that need to be destroyed. Sterilization Protocol B is designed (1) to be used on a sample which has been more thoroughly examined and shown no evidence of biohazards in testing against representative samples from a variety of plants and animals, and (2) not to harm the sample for most purposes of geochemical testing. An example of a Protocol B might be exposure of the sample to 20-30 megarads of gamma radiation. It should be

assured that any scientist studying the samples after sterilization will take standard chemical safety measures against small molecules (carcinogens, teratogens, poisons).

The entire process of testing and sterilization should be carefully validated using Earth samples. In particular, the combination of dry heat and irradiation or irradiation alone needs to be examined using dry organisms in rocks similar to those from Mars and from fragments of martian meteorites. The hierarchy of tests needs to be scrutinized for assay sensitivity, sampling statistics, method reproducibility, and operator expertise.

Note: The process suggested by the Sub-group makes no correlation between each set of tests and the level of biosafety required. Most tests will be run under BSL-4 conditions, but samples tested as negative in Test Set B might be subjected to some of the tests in Set C under BSL-3 conditions.

Protocol For Sample Distribution

Assumptions

- Risk Assessment
  - Viruses are not likely to exist in absence of hosts on Mars
  - Organism(s) growing on Mars unlikely to colonize terrestrial hosts
  - Life forms as we know them

- Sterilization is effective
  - Irradiation will kill self-replicating life forms (>10^8 Daltons)
  - Inorganic template too improbable to be a real hazard
  - 20-30 Mrads OK for geological analysis, study of smaller organics
  - If life form cultured, products can be used to test sterilization protocol

- Standard chemical safety measures against small molecules (carcinogens, teratogens, poisons) will be taken by user

- Need to look at changes using Earth samples
  - Look at irradiation + dry heat sterilization protocols
  - Determine effects on organisms of protocol in dry, solid matrices
  - Run through hierarchical protocol with terrestrial organisms to test:
    - Assay sensitivity
    - Sampling statistics
    - Method reproducibility
    - Operator expertise

Sub-group 2C

Issue
Can sub-samples of preserved martian meteorites serve as models ultimately to test sterilization methods, procedures, and effectiveness?

The members of the Sub-group were:
Robert W. Walker (Chair)
Carlton C. Allen
Heinrich D. Holland
Gerhard Kminek
Dimitri Papanastassiou

Answer
Yes, but…

Reasoning
Yes, samples selected of appropriate martian meteorites should be used to test sterilization procedures, **but** only after the procedures have been fully developed and tested on terrestrial analogs.

Using actual martian samples has two advantages:

1) The sterilization procedures would be tested as realistically as possible.

2) The tests would determine the effects of proposed sterilization procedures on the mineralogy and petrography of martian rocks themselves.

Martian meteorite samples are precious and should not be destroyed cavalierly. Therefore, the final tests should use only limited quantities of material. We suggest, subject to the approval of the Meteorite Working Group (MWG, which is responsible for the allocation of Antarctic meteorites), that Antarctic specimens might be best suited for this purpose. Specifically, we recommend that a small sample of ALH84001 - the meteorite in which some individuals have reported evidence of putative fossil life - be made available for this study. The investigations should not be confined to the biological tests alone. They should also include measurements designed to assess the deleterious effects of the sterilization procedures themselves. In this regard, samples of an additional martian meteorite (not necessarily an Antarctic meteorite) containing low temperature mineral phases should also be studied.

One possible protocol, among many others, would be to divide the sample into three aliquots. One would be kept as a control and a reserve. The second aliquot would be subjected to sterilization procedures directly. The third would be deliberately dosed with known terrestrial organisms and then sterilized and tested.

Sub-group 3A

Issue
Given the uncertainties, what are effective sterilization methods for samples returned from Mars?

The members of the Sub-group were:
Carl W. Bruch (Chair)
John Battista
Daniel Branton
Harry Crissman
James G. Ferry
Dean W. Gabriel
Jacques Grange
Ellis Kempner
Gerhard Kminek
Frances S. Ligler
Irving Pflug
Robert R. Reich
David Stahl
Eric Stanbridge
Jonathan D. Trent

Discussion

Validation is possible. Using the assumptions presented by Sub-group 2B (see p. 27) as a basis, the Sub-group formulated the following parameters in order to make recommendations for sterilization methods pertaining to samples returned from Mars. The parameters include the concept that sterilization techniques can only be assessed in terms of dosing and effectiveness based upon our existing knowledge of Earth organisms. This knowledge provides the basis with which to assign or derive dosage levels for sterilization. Initially, these dosages should be based on an "overkill" approach to ensure maximum safety levels for humans and the environment. At the same time, another parameter of utmost importance is the requirement to maintain sample integrity in developing the required protocols. The protocols must be minimally destructive to the sample so that future scientific analyses are not compromised.

In the context of what is currently known about sterilization of resistant organisms (viruses, bacteria, prions etc.), the Sub-group reviewed a number of known sterilization techniques that would be suitable for application to martian samples. These techniques included ethylene oxide gas sterilization, radiation, dry heat, and combinations of radiation and dry heat. For example, the synergistic killing effect from the combination of dry heat with ionizing radiation was acknowledged, along with the deleterious effects that this process could have on the geological and chemical composition of samples. While radiation and heat were considered to be the methods of choice, doses need to be determined as well as compositional impacts on the sample. Penetrating methods of sterilization (i.e., heat, radiation) are more likely to be effective than surface methods (ethylene oxide, chemical washes).

Based upon current medical practices, the Sub-group determined that the clinical sterility assurance level (SAL) of a 10^{-6} probability of survival is a reasonable SAL for martian samples. In order to demonstrate reproducibility for the D_{90} value, and in order to reach the specified SAL for the proposed sterilization doses, multiple analytical runs should be implemented on various substrates that mimic the martian samples to be returned. In turn, martian samples could be split to minimize the fraction of sample that would be subjected to destructive sterilization techniques.

Proposed Test Sets

The Sub-group then reviewed the sample distribution recommendations from Sub-group 2B (see p. 25) and proposed specific sterilization parameters associated with each designated Test Set. The parameters for each test set are as follows:

Test Set A

Test Set A (see Chart 1, p. 30) is defined to be the most rigorous set of sterilization conditions. This test set focuses on an "overkill" sterilization model based upon the most resistant organisms (viruses) that are currently known. In order to assure absolute sterility, radiation and dry heat were recommended. The suggested ionization dose of 50 Mrads is based on 20 times the European Pharmacopoeia. A simultaneous dry heat temperature of 120ºC for approximately 24 hours would provide a synergistic effect on the sterilization. It is recognized that these parameters would cause significant deleterious effects to the samples, therefore compromising their value to geologists and chemists. The Parvovirus is suggested as a test organism.

Test Set B

Test Set B (see Chart 2, p. 30) represents a less stringent protocol for sterilization and is based on cells, and uses bacteria as the standard. The recommendation is a lower dose of ionization radiation applied to cold, dry samples. Radiation resistant organisms such as *Deinococcus radiodurans* and phage (M13) are suggested for modeling purposes.

Test Set C

Test Set C allows distribution of samples to the scientific community without sterilization. These samples are maintained in the most pristine state possible, and may be subject to containment requirements.

Based on Test Sets A-C, as defined, the Sub-group revised a sample distribution model. The first revision illustrated in Figure 1 (see p. 31) was expanded to yield the final model shown in Figure 2 (see p. 31). This model associates the level of hazard with each Test Set, so that specific risk factors are associated with the test requirement (i.e., Test Set A, human hazard; Test Set B, animal and plant hazard; and Test Set C, environmental hazard).

CHART 1: Proposed Test Set A

Sample Prep:
approximately 1 gram

Test Organisms:
Viruses, particularly Parvovirus

Proposed Assays:
Human cell culture

Sterilization techniques:

Ionizing Radiation

Dose level: 50 Mrads*

. Recommend that this is performed simultaneously with the dry heat step. This should produce a synergistic effect that will reduce the required amount of either technique

Dry heat (simultaneous with ionizing radiation)

Conditions based, in part, upon European Pharmacopeia:
120ºC for 40-50 hours
140ºC for approximately 4 hours
160ºC for 30 minutes

*All sterilization conditions proposed will need to be refined with follow-up models.

CHART 2: Proposed Test Set B

Sample Prep:
approximately 1 gram

Test Organisms:
Bacteria, including *D. radiodurans*
Phage: (M13)

Proposed Assays:
Cell Culture

Chronic *in vivo* study in mammalian model system

Sterilization technique:

Ionizing Radiation

Dose: 20 Mrads
Conditions: cold, dry
Model: terrestrial samples

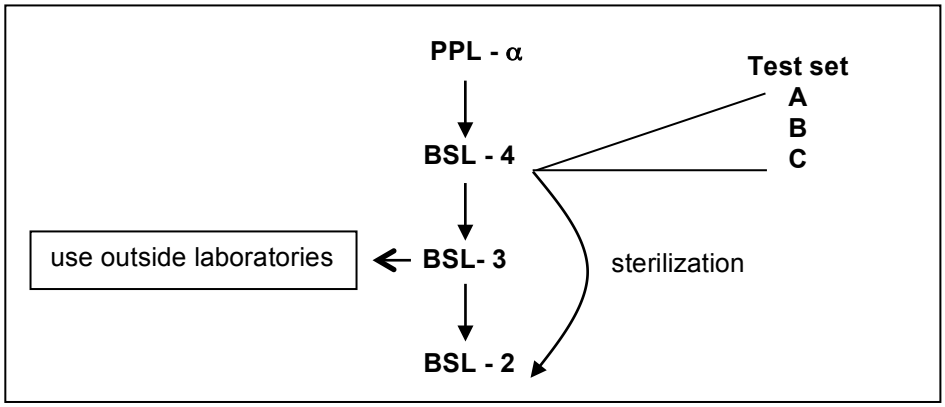


Figure 1. Laboratory Categorization

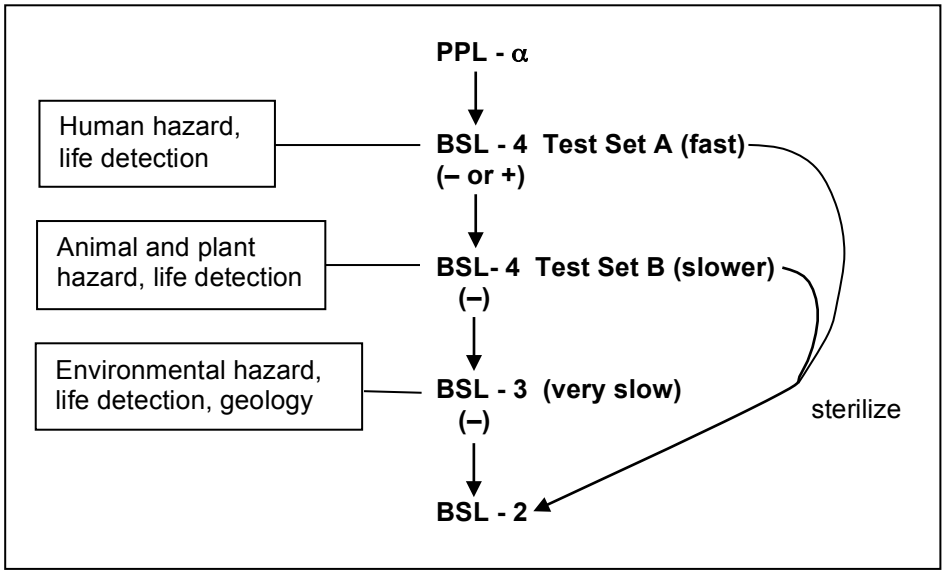


Figure 2. Proposed Model

Sub-group 3B

Issue

Given the uncertainties, what are effective sterilization methods for samples returned from Mars?

The members of the Sub-group were:

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Paul Brown
Robert Rohwer
Virginia C. Chamberlain
Michael J. Daly
Martin Favero
William N. Fishbein
Heinrich Holland
Joseph B. Lambert
Dimitri Papanastassiou
Ronald F. Schell
Peter Sheridan
Indra K. Vasil
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John Williams

Preamble

The chemical elements on Mars are the same as on Earth, and the forces holding molecules together are also the same. If there were a life form on Mars based on other than carbon-containing molecules, the energies holding such molecules together would not be much different than those for proteins and polynucleotides. Hence, bond breakage by heat or gamma radiation should be similar on Earth and Mars, and sterilization conditions for Earth microorganisms should eradicate microorganisms of similar size from Mars.

There is no absolutely optimal approach, **but…**
Enough is known about the relationship between organism size, repair mechanisms, and survivability, that the maximum survivability of "designer" martian organisms can be estimated with some confidence.

Effect of radiation on geochemistry

Many of the key parameters measured by geochemists are unaffected by sterilizing gamma doses. (Allen et al., 2000) Gamma photons from ^{60}Co (1.17 – 1.33 MeV) in doses as high as 30 Mrad do not induce radioactivity in rock and mineral samples. Such doses also produce no measurable changes in isotopic compositions, elemental compositions, or crystallographic structures. The only detectable effects are changes in albedo, color, and thermoluminescence in selected minerals.

Isotopic and elemental compositions will not be affected regardless of gamma dose. Sterilization at doses significantly above 30 Mrad may induce changes in crystallographic structure (**caveat:** research required) and dose-dependent changes in albedo, color and thermoluminescence are expected.

On balance, if samples returned from Mars require biological sterilization, exposure to gamma rays or high energy electrons provides a feasible option.

Sterilization

Aim: The aim of the sterilization procedure is to reduce the risk of "significant adverse effects" of samples distributed to the scientific community. These levels are defined to be such that the adverse

effects on humans, animals, and the environment is less than 10^{-6} (i.e., that less than one sample in 1 million distributed to the scientific community will produce a significant adverse effect).

Procedure: The suggested procedure for sterilization consists of irradiation with gamma rays at temperatures up to approximately 105°C. This procedure has the advantage of being able to kill all known terrestrial organisms while probably doing minimal damage to the non-biologic constituents of the Mars samples.

Conditions of irradiation: The survival rate of a large number of terrestrial organisms exposed to ^{60}Co gamma rays has been determined as a function of dosage, dose rate, and temperature.[2] There are no terrestrial organisms known whose probability of survival is $>10^{-6}$ at a dose of 20 Mrad at room temperature. However, populations of individual organisms may require higher doses to ensure that the probability of finding any survivor is $<10^{-6}$. The survival rate at a given total dose decreases with increasing temperature during irradiation. For example, the sensitivity of dry T1 bacteriophage to inactivation by X-rays increases, or the D_{37} decreases by ~10-fold between 60 and 105ºC (Pollard, 1953).

If martian organisms returned to Earth are similar to terrestrial organisms, a dose of 20 Mrad at 105°C should reduce their number to $<10^{-6}$ of their initial number. It is not clear, however, that martian organisms are similar to terrestrial organisms. It is possible, for instance, that they are much more resistant to gamma radiation. A good deal is known about the relationship between the size and the biochemistry of terrestrial organisms and their resistance to gamma radiation. It has been shown that smaller organisms tend to survive higher radiation doses,[2] and the strategies used by microorganisms to increase their resistance to radiation are not all understood. It might therefore be a useful exercise to explore hypothetical possibilities for the evolution of martian organisms adapted to the much higher radiation fluxes to which they would be subjected, compared to terrestrial microbes. The radiation dose at various temperatures required to reduce the probability of the survival of such organisms below 10^{-6} per sample could then be estimated and could become the basis of irradiation protocols for the sterilization of returned Mars samples.

It is possible, though very unlikely, that martian organisms are not carbon based.[2] A thorough analysis of the possibility that martian biology is based on other elements (Si, N, P, O, H, S, Al, and B come to mind) should be carried forward. Si-based "life" on Earth has been suggested for the clay minerals. It is a moot question whether clays should be considered "alive." It is clear that they do not represent a health or environmental danger on Earth.

Sterilization Conditions

First, we note that a large number of geochemical tests will be carried out in the maximum containment facility on arrival of the specimen. These tests will likely include X-ray tomography to determine loci of cracks & other separations where life-forms would be most likely, and Total Organic Carbon (TOC), which permits a limit on the density of C-C organisms. Ignoring the chemical basis of any life-form, we can provide a confidence level of sterilization, with only 2 assumptions: 1) Any reproducing life-form must be based on macromolecules (i.e., polymers) with interatomic covalent bonds (not crystal lattices). 2) Since all such bonds have similar strength, destroying these destroys the life-form.

2 See Supplement to Sub-group 3B: Sterilization by Ionizing Radiation, p. 35.

Evidence shows that 55 Mrads radiation will destroy almost all known bacteria, viruses, spores, and prions (e.g., causative agent of Scrapie) by 1 million-fold. Using 100 Mrads would give a 10-fold safety margin. If worst-case estimates are used (10^6-10^{12}/gm of martian sample and a tiny target, such as a virus) sterilization would require 400 Mrads.[3] Even this may be satisfactory for most geologic studies (100 Mrads is OK). This amount of radiation could be safely reduced if the irradiation were carried out at elevated temperature, and/or if the TOC is low enough.

Experiments Needed

There is a need for more experiments to serve as a basis of information on how to deal with martian samples. First, there are a number of experiments that should be done using materials and microbes on Earth (Pre-sample Studies). Following a return mission, there should be a specific set of experiments planned for the samples (Post-sample Studies).

The following Pre-sample studies would be useful in guiding the development of sterilization protocols:

- Seek out and study microorganisms growing in high radiation environments, such as in nuclear reactors.
- Collate existing data and obtain new data on extremophiles and traditional microbial systems. Obtain estimates of required sterilization doses for heat/radiation sterilization.
- Determine what are the most difficult organisms to sterilize by these methods, looking particularly at traditional sterility testing of microbes/spores, extremophiles, fungi, viruses, and prions.
- Carry out sterilization experiments on simulated martian soil/rocks. Then with martian meteorite material.
- Study the bond energies of non-carbon polymers. Study the limits on nucleic acid repair. These studies could be used to justify ignoring the possibilities of non-carbon based life in the development of sterilization protocols.
- Examine current hazard containment methods.
- Test interference, sensitivity, sampling, and extraction techniques on simulated martian rocks.
- Compare sterilized and unsterilized martian meteorite material to examine the feasibility of geological studies after sterilization.
- Find the upper limit on the number of bacteria that can exist on rock material. This can be estimated based on Total Organic Carbon (TOC).

The following Post-sample studies would be useful in determining the requirements for the sterilization procedures that will be used:

- X-ray tomography, TOC, and MS analyses. Although these studies may not necessarily prove or preclude the existence of life in the samples, they should reveal, at the least, the maximum number of microbes that could be present in the samples.
- If life is found, we will need survival studies on the organisms isolated, in order to refine the dose needed to sterilize.

3 ibid.

Conclusion

The best approach to assure destruction of known life forms may be a combination of gamma ray or high energy electron exposure plus simultaneous dry heat. The exposures and temperatures should be determined experimentally starting with data from the effects of radiation and heat delivered separately. The required conditions are likely to be >10Mrad and >95^{o}C, and should include experiments on prions.

Supplement to Sub-group 3B: Sterilization by Ionizing Radiation

Robert Rohwer

Assuming first order inactivation kinetics and no absorbance of the radiation by the sample matrix, the sterilization potential of ionizing radiation can be predicted from the expression $S = e^{-D/D37}$ where:

S = surviving fraction

D = dose in rads

D_{37} = dose required to inactivate l/e of the original population

The dose required to inactivate any given organism to the 10^{-6} survival level depends on its D_{37} and its concentration in the sample. A worst case computation based on terrestrial organisms would utilize the highest known concentrations of organisms in combination with the highest known D_{37} values found for Earth bound organisms. The highest D_{37} values are those of *Deinococcus radiodurans*, smaller viruses, and transmissible spongiform encephalitis (TSE) agents. Organismic concentrations vary from several hundred organisms per gram of ice core or extreme soil to >10^{8}/g in productive soils to 10^{14}/g in stools or yogurt. These conditions and the doses required to reduce survival to 10^{-6} have been computed in Table 1.

Table 1. Dose of ionizing radiation required, at room temperature, to reduce viability of organisms to 10^{-6} as a function of D_{37} and organism concentration.

Organism	$Dose_{37}$ of the Organism	Concentration of Organisms No./g		
		1	10^6	10^{12}
		Total Dose in rads		
Small viruses	10^6 rads	14 x 10^6	28 x 10^6	41 x 10^6
Deinococcus Radiodurans (anoxic wet)	1.8x10^6 rads	25 x 10^6	50 x 10^6	75 x 10^6
Prions	4 x 10^6 rads	55 x 10^6	110 x 10^6	170 x 10^6
Circo virus (estimated)	10^7 rads	140 x 10^6	280 x 10^6	410 x 10^6

$S=e^{-D/D37}$ where S = fractional survival after exposure to dose, D, when the dose required to inactivate 1/e is D_{37}. Rearranging gives D = D37 x ln(S).

It is evident from Table 1 that an absorbed dose of 410 Mrad provides a high level of security against all recognized terrestrial organisms. Provided that martian life is based on chemistry similar to that for Earth organisms, it seems likely that this dose would also provide a high level of security against martian life. Nevertheless, since little if any radiation inactivation data has been collected under the specific conditions that will be encountered in this project, it will be essential to validate any protocol that is adopted. Simulations will need to be as realistic as possible using a broad spectrum of the most challenging organisms and molecules.

More research is needed. It is essential that a comprehensive effort be made to investigate the most resistant terrestrial species – including organisms that are reputed to colonize the fuel in waste reactor elements and other high exposure environments. Special attention, including research support, should also be given to the development of better methods for culturing and characterizing currently unculturable organisms. These unculturable organisms may represent the majority of naturally-occurring terrestrial organisms. Attention should also be given to the potential for stabilizing effects of the intimate matrix associations that must be postulated in the case of organisms associated with the interior of rock or mineral specimens. It is recognized that even in the case of ionizing radiation, inactivation kinetics do not always take the form of first order kinetics. Explanations for such deviations are not always apparent, but where deviations occur, large excursions from expectation are possible.

Supplement to Sub-group 3B: Life Forms Based on Silicon Polymers

Joseph Lambert

Life, as we know it, is based on molecules built primarily from carbon-carbon (C-C) bonds, with a few carbon-oxygen (C-O) and carbon-nitrogen (C-N) bonds. The molecules of life have structural, catalytic, metabolic, and informational purposes. Thus, some serve to provide structure to life forms, some control the chemical reactions that construct and maintain these structures, others provide energy to drive these processes, and still others contain and manipulate the information required to maintain the accuracy of these chemical processes through reproduction. These biomolecules include amino acids, sugars, nucleotides, lipids, and a few other classes. When combined with other like molecules into larger, more organized and more information-rich forms, they become proteins, polysaccharides, and nucleic acids.

Despite the diversity of these molecules, they have many common characteristics, including the exclusive use of carbon as the building block. Mostly frequently, the skeletons of these molecules are composed of C-C bonds. The C-O bond features prominently in sugars and the C-N bond in proteins and nucleic acids. Thus, it has become a common expectation that the element carbon is a necessity for life of any kind. In the periodic table, the element most closely resembling carbon is silicon (Si), its next neighbor in the same vertical group or family. Consequently, it is reasonable to ask whether Si could substitute for C in biomolecules, and whether life forms might even draw primarily on Si in a carbon-poor environment. Indeed, Si is the second most abundant element (after oxygen) in the lithosphere of our planet and is likely to be among the most abundant of elements on Mars, or any planet that is composed largely of solid rocks. The primary bond in siliceous rocks is between silicon and oxygen (Si-O).

Nonetheless, there is essentially no likelihood that life could be based on siliceous molecules. Despite its considerable abundance on Earth, evolutionary forces ignored silicon and instead utilized its much less abundant neighbor, carbon. In the first place, the bonds between two silicon atoms (Si-Si) are easily broken by light, acids, bases, and even heating. Life apparently requires solvents and the liquid state. Yet, temperatures that permit fluidity easily destroy polysilanes, as such molecules are called. They would be an extremely unreliable material to serve as the basis of life.

Possibly even more important, all classes of terrestrial biomolecules contain double bonds between atoms, with C=C, C=O, and C=N bonds found commonly in peptides, nucleotides, and even sugars. Indeed, the chemical group that generates the acidity of amino acids (the carboxyl group, CO_2H) contains the C=O bond. The link between every peptide unit in polypeptides contains the C=O bond and a partial C=N bond. All genetic information comes from the so-called bases in the nucleic acids (purines and pyrimidines), which are composed primarily of C=C, C=O, and C=N bonds. Most of the chemical reactions that create biomolecules and control their reproduction require double bonds. Thus, it is a fatal limitation to the concept of silicon-based life that double bonds to Si essentially do not exist. To date, there is no known molecule with a stable Si=O bond, and molecules containing Si=C, Si=Si, and Si=N bonds have only been made in the last few years in the laboratory. These molecules do not exist in nature, and they require very special conditions to be at all stable in the laboratory. The low stability of double bonds to Si results from the fact that any element of higher atomic number must have longer bonds because of its larger atomic size. The second bond in all double bonds is very dependent on the distance between the atoms: the greater the distance, the weaker the double bond.

Lacking double bonds, silicon is essentially incapable of providing an important role in life. On Earth, it is relegated biologically to a few examples of structural molecules made up of Si-O single bonds, the same types of bonds found in silicate minerals.

Carbon-based life would have been essentially impossible with only single-bonded molecules. It is impossible to imagine life based entirely on simple alkanes, which comprise the class of carbon compounds containing only single bonds. Analogously, life cannot exist with Si as the primary building block, although Si is a superb contributor to the rocks of the inorganic world and, in its elemental form, to electronics. Modern synthetic chemists have designed polymers (such as silicone rubber) that contain Si-O bonds as the primary skeletal component and Si-C bonds as modifiers attached to the Si-O skeleton. All bonds in these versatile materials are single bonds. Like the silicates of geology, the silicones of industry have varied properties, but cannot provide the functions of life. Silicone polymers lack the versatility and information content that is provided by double bonding.

APPENDIX A:
WORKSHOP 2a AGENDA

Tuesday, 28 November 2000

0830	**Plenary Session: Introduction, Overview & Task Assignments** Mars Planning Overview, and Introduction of the Co-Chairs	John D. Rummel
0845	**Plenary Session: Microbial resistance and biological control**	Richard Setlow *Co-Chair*
	• The environment (radiation and temperature) on Mars	Richard Setlow
	• Quantitative effects of ionizing radiation on proteins and viruses	Ellis Kempner
	• Effects of radiations and desiccation on microorganisms	John Battista
	• The detection of microorganisms	Ronald Schell
0945	**Plenary Session: Sterilization methods and NASA applications**	Carl Bruch *Co-Chair*
	• Brief overview of sterilization methods	Carl Bruch
	• Planetary quarantine spacecraft sterilization and sample return issues	Martin Favero
1045	Break	
1100	**Morning Sub-groups**	

Issue 1A: Can survival mechanisms of terrestrial extremophiles serve as models of how putative martian extremophiles might exhibit resistance to sterilization?

Issue 1B: Given (a) the range of uncertainties regarding putative martian life's origins, evolution, and adaptation to extreme environments, (b) our limited knowledge of the biological evolutionary potential on only one planet, and (c) terrestrial biohazard mechanisms of action, what is the worst-case scenario for sterilizing martian samples?

Issue 1C: What sterilization methods and procedures will best preserve the integrity of martian soil and rock samples for future scientific analyses outside the proposed containment facility?

Issue 1D: If life detection initiatives do not yield evidence of carbon or polymers, is sterilization of representative martian soil and rock samples necessary prior to distribution to awaiting scientists outside the containment facility?

1230	Working Lunch (in Sub-groups)
1330	Sub-group discussions continue
1430	Plenary Session: Summaries from Day 1 Morning Sub-groups
1530	Break

1545 Afternoon Sub-groups
Two Independent Sub-groups
Issue 2A/2B: Given (a) the uncertainties about putative extraterrestrial life in extreme extraterrestrial environments, (b) our limited knowledge about the evolution of sterilization resistance mechanisms in terrestrial extremophiles, can martian samples be sterilized effectively and safely distributed outside the containment facility before life detection, biohazard testing, and other chemical analyses are actually completed and evaluated?

One Sub-group
Issue 2C: Can subsamples of preserved martian meteorites serve as models ultimately to test sterilization methods, procedures, and effectiveness?

1800 Sub-groups Adjourn, Day 1

1830 Reception

Wednesday, 29 November 2000

0800 Day 1 Afternoon Sub-groups Reconvene

0830 Plenary Session: Summaries from Day 1 Afternoon Sub-groups

0930 Day 2 Sub-groups

Two Independent Sub-groups
Issue 3A/3B: Given the uncertainties, what are effective sterilization methods for samples returned from Mars?

1230 Lunch

1330 Sub-groups Reconvene

1500 Plenary Session: Day 2 Morning Sub-groups Report

1700 Plenary Session: Sub-group Writing Assignments

1800 Adjourn, Day 2

Thursday, 30 November 2000

830 Plenary Session: Summaries from Day 2 Sub-groups

1000 **Plenary Session: Group Discussion**
Task: Resolve differences regarding task issues on validation and assurances of effective sterilization, in accordance with developing applicable portions of the protocol.

1100 Adjourn

APPENDIX B1:
WORKSHOP 2a PARTICIPANTS' AREA(S) OF EXPERTISE

Name	Affiliation	Area(s) of Expertise
Allen, Carlton C.	NASA Johnson Space Center	Sample handling and curation; physical/Earth and planetary sciences
Baker, Amy	Technical Administrative Services	Technical editor
Battista, John	Louisiana State University	Bacterial DNA repair and mutagenesis
Beaty, David	NASA Jet Propulsion Laboratory	Mars science and advanced missions
Benner, Steven A.	University of Florida	Bioorganic and bioinorganic chemistry
Branton, Daniel H.	Harvard University	Single nucleic acid molecule detection; nanopore sequencing methods
Brown, Paul	National Institute of Neurological Disorders and Stroke	Prion diseases
Bruch, Carl W.	Formerly, U.S. Food and Drug Administration	Sterilization methods and regulations
Chamberlain, Virginia C.	V. C. Chamberlain and Associates	Sterilization/quality systems
Crissman, Harry A.	Los Alamos National Laboratory	Flow cytomery and cytochemical life detection methods
Daly, Michael J.	Uniformed Services University	Radiation resistant bacteria
Favero, Martin S.	Johnson and Johnson	Sterilization methods
Ferry, James G.	The Pennsylvania State University	Functional genomics and transcriptional regulation in Archaea
Fishbein, William N.	Armed Forces Institute of Pathology	Toxicologic pathology
Gabriel, Dean W.	University of Florida	BSL-3 plant pathogen containment; molecular plant pathology
Grange, Jacques	Laboratoire de Haute Securite P4 Jean Merieux	Responsible for the MERIEUX BSL-4 Facility; biochemistry, cancer research, and virology
Holland, Heinrich D.	Harvard University	Ecology; atmospheric chemistry
Kempner, Ellis	National Institutes of Health	Macromolecular biophysics; prions
Kminek, Gerhard	University of California-San Diego	Chemistry and evolution; evolution of chemical systems
Lambert, Joseph B.	Northwestern University	Polymer chemistry of silicon and other main-group elements; self-assembly and synthesis of novel molecules with unusual electronic properties
Ligler, Frances S.	U.S. Naval Research Laboratory	Microbial immunoassay life detection methods
Lindstrom, David J.	NASA Johnson Space Center	Geochemistry; curation of extraterrestrial samples
Macke, Jennifer	Science and Technology Corporation	Technical editor
Papanastassiou, Dimitri	NASA Jet Propulsion Laboratory	Geochemistry

Name	Affiliation	Area(s) of Expertise
Pflug, Irving	University of Minnesota	Dry heat and ethylene oxide sterilization applications to *Viking*
Reed, Craig	U.S. Department of Agriculture	Food safety
Reich, Robert R.	Pharmaceutical Systems, Inc.	Developing ethylene oxide biological indicators for industrial and hospital applications
Rohwer, Robert	VA Medical Center at Baltimore; University of Maryland at Baltimore	Molecular neurovirology; prion diseases; inactivation of pathogens
Rummel, John	NASA Headquarters	Workshop Planning Committee member
Schad, P. Jackson (Jack)	U.S. EPA; NASA Headquarters	Workshop Planning Committee member
Schell, Ronald F.	University of Wisconsin	Rapid fluorescent methods to detect bacterial pathogens
Setlow, Richard B.	Brookhaven National Laboratory	Biophysics; radiation biology
Sheridan, Peter	Pennsylvania State University	Psychrophilic, cold-loving microorganinsms; biochemical characterization of cold-active enzymes
Stabekis, Pericles D.	Lockheed Martin	Workshop Planning Committee member
Stahl, David A.	University of Washington	Molecular evolution of complex anaerobic microbial ecosystems
Stanbridge, Eric J.	University of California	Molecular detection of microorganisms in clinical settings; cancer etiology
Trent, Jonathan D.	NASA Ames Research Center	Extremophiles
Vasil, Indra K.	University of Florida	Plant tissue culture and biotechnology
Wahl, Beth	SAIC	Contracts administrator
Walker, Robert M.	Washington University in St. Louis	Geophysics; lunar samples and meteorites; effects of radiation on physical properties of solids
Williams, John L.	U.S. Department of Agriculture, Animal and Plant Health Inspection Service	Background in military research; veterinarian
Wisniewski, Richard S.	NASA Ames Research Center	Biotechnology; Pharmaceutical and biopharmaceutical process development

APPENDIX B2:
PARTICIPANTS' CONTACT INFORMATION

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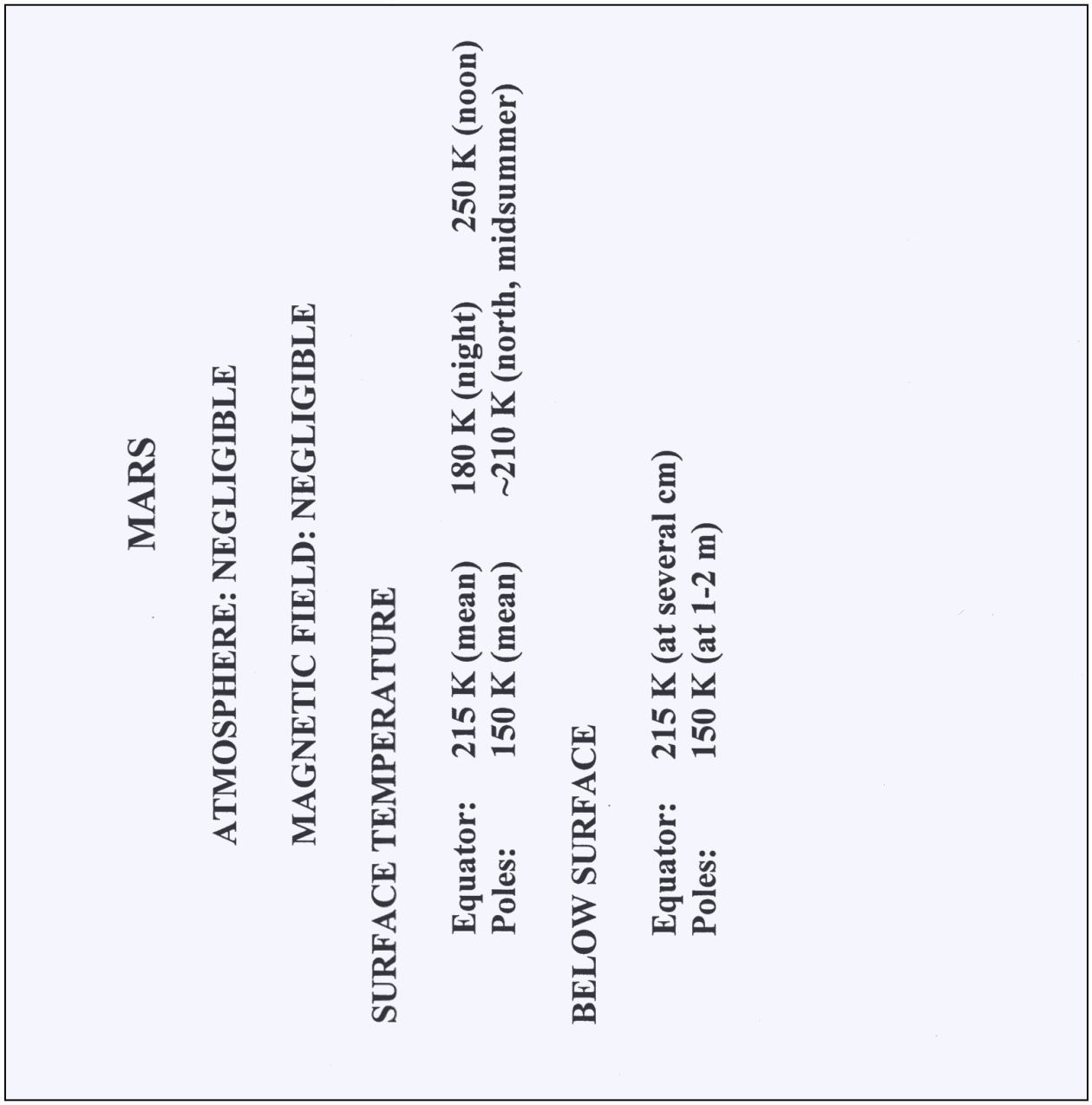
APPENDIX C1:
PLENARY PRESENTATION MATERIALS
Richard Setlow



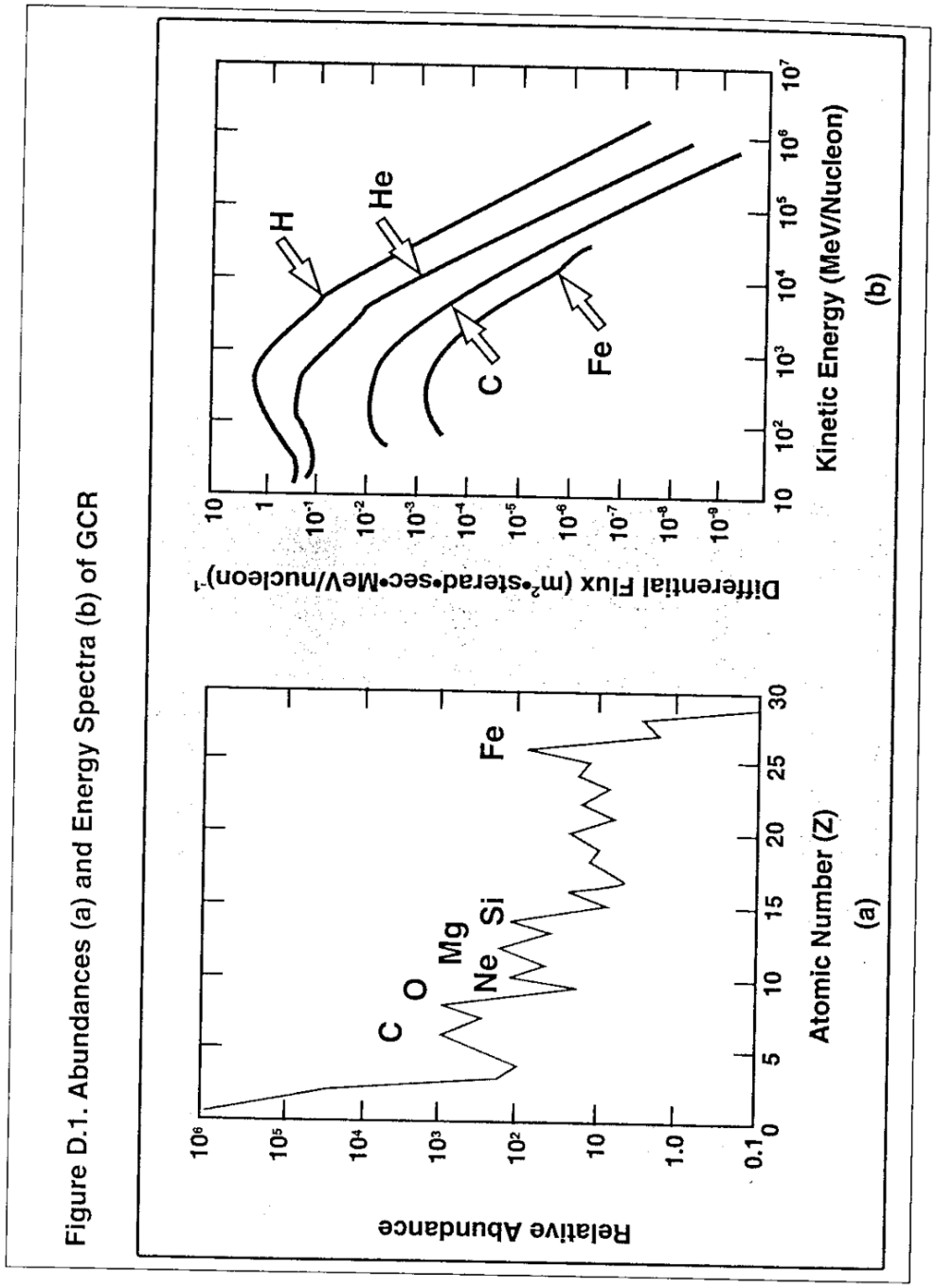


Figure D.1. Abundances (a) and Energy Spectra (b) of GCR

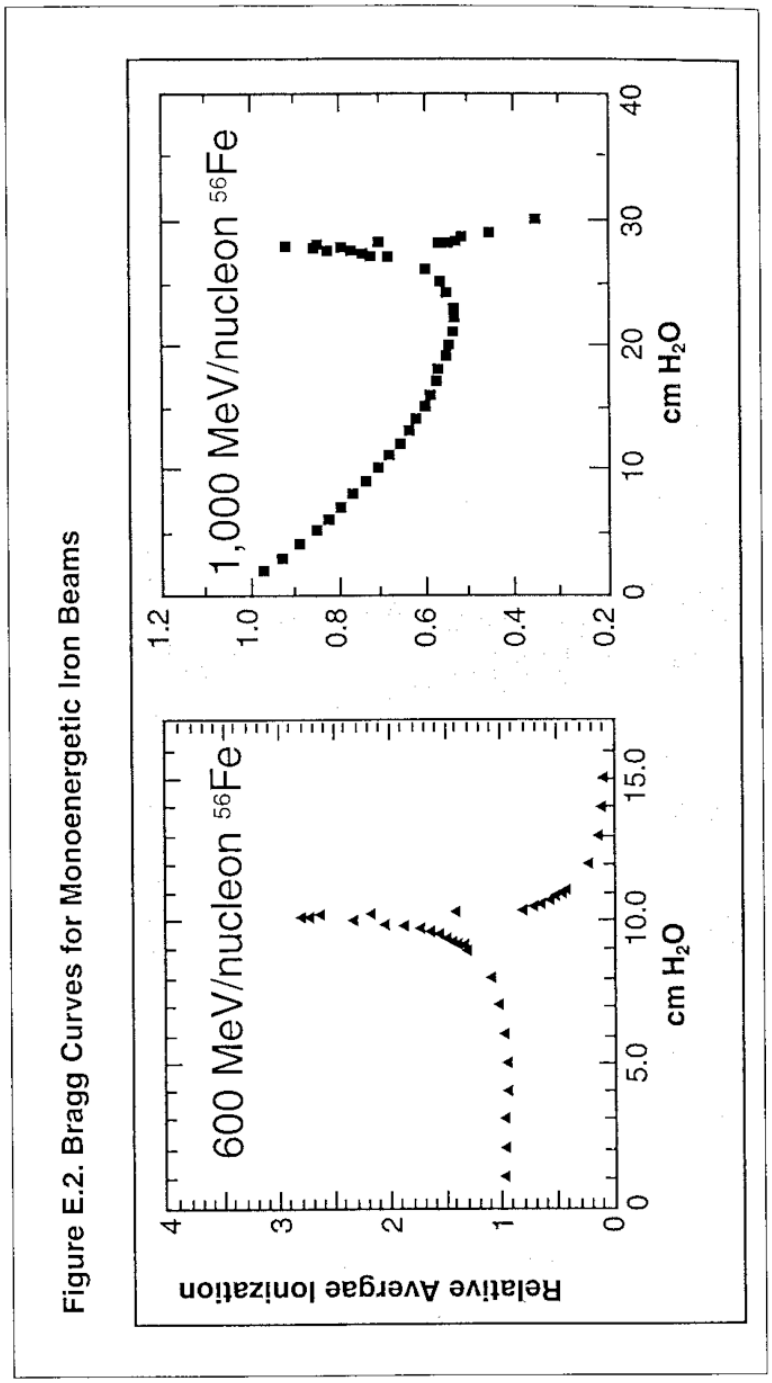
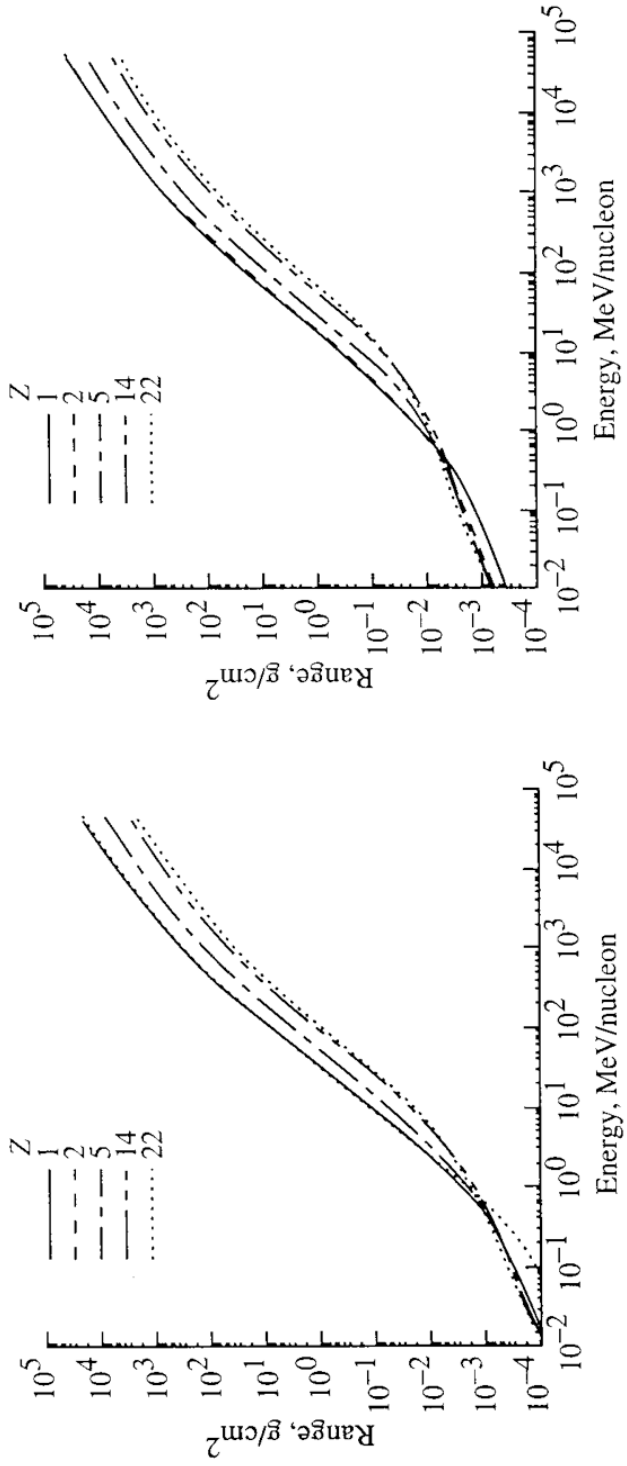


Figure E.2. Bragg Curves for Monoenergetic Iron Beams



Stopping ranges of selected ions

ESTIMATED RADIATION EXPOSURE DURING A TRIP TO MARS AND RETURN

Particle	Dose	Hits per Nucleus
Protons	250 mGy	400
α-particles	35 mGy	40
HZE-particles (Z from 3 to 28)	3 mGy	3
Solar Particle Events	~1 Sv	

APPROXIMATE FLUENCES AT ~290 nm THAT WILL INACTIVATE 63% OF THE FOLLOWING

E. Coli	**13 J/m^2**
T4 phage	**7**
ΦX174 phage	**30**
Dry trypsin (300K)	**$7x10^4$**
Dry trypsin (90K)	**$15x10^4$**
Prions (est)	**$20x10^4$**

SUNLIGHT ABOVE THE EARTH'S ATMOSPHERE AT ~290 nm

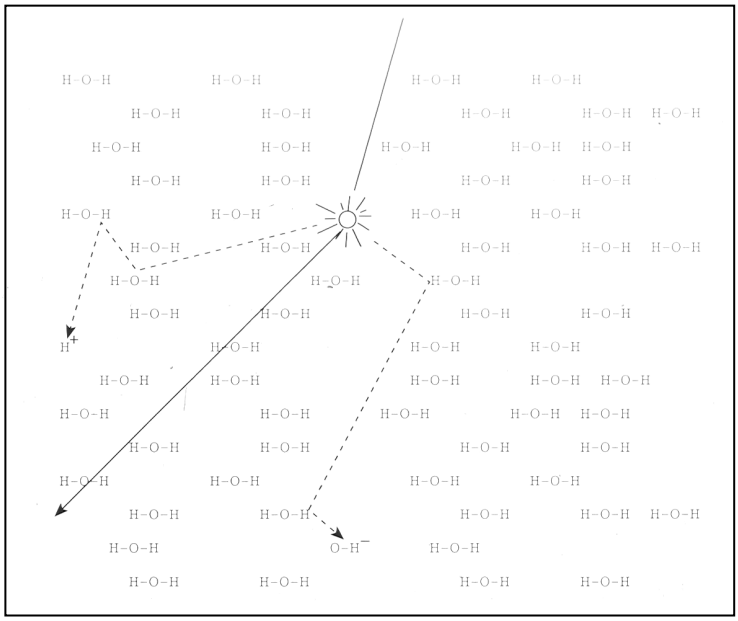
10 W/m^2 = $4x10^5$ J/m^2/24 hours

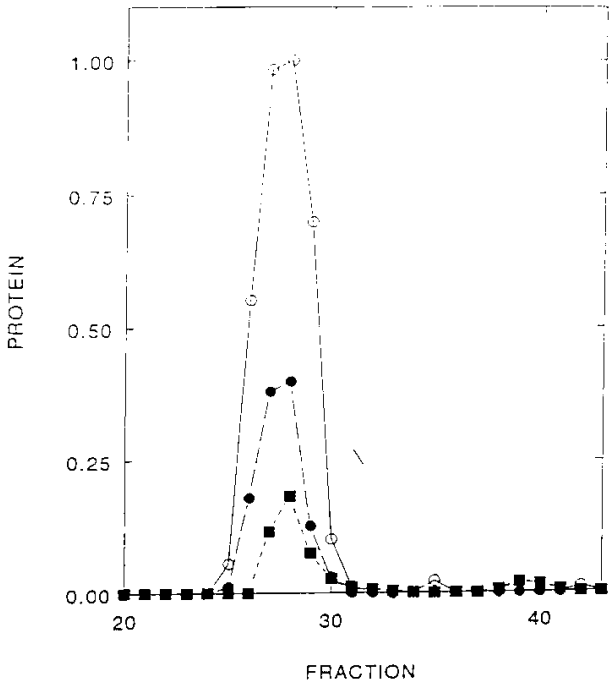
AT THE SURFACE OF MARS

$18x10^4 J/m^2$/24 hours

rbs 10/16/00

APPENDIX C2:
PLENARY PRESENTATION MATERIALS
Ellis Kempner





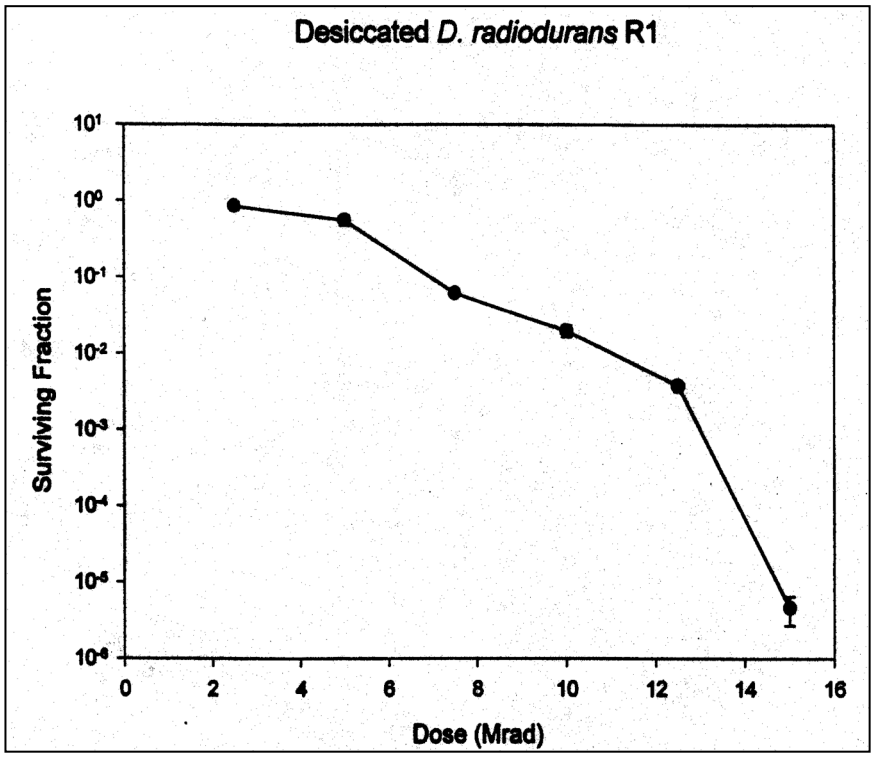
Effect of radiation on glucose-6-phosphate dehydrogenase.

APPENDIX C3:
PLENARY PRESENTATION MATERIALS
John Battista

D_{37} Dose for Representative Cell Types

Most Normal Human Tissue	**0.8 Gy**
HeLa Cells	**1.3 Gy**
E. coli	**30 Gy**
***Bacillus megaterium* spores**	**1000 Gy**
Tobacco Mosaic Virus	**4000 Gy**
***Deinococcus radiodurans* R1**	**6500 Gy**

1Gy = 100 Rads


Desiccated D. radiodurans R1
Surviving Fraction
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
10⁻⁶
0
2
4
6
8
10
12
14
16
Dose (Mrad)

Radioresistant Microorganism

- Radioresistance cannot be an adaptation (i.e., an evolutionary modification of a character under selection) to ionizing radiation, because there is no selective advantage to being ionizing radiation resistant in the natural world.
- There are no terrestrial environments that generate a high flux of ionizing radiation.
- It must therefore be assumed that ionizing radiation resistance is an incidental characteristic.

Deinococcus radiodurans

- Survives desiccation remarkably well for a vegetative organism. In one anecdotal report, this species exhibited 10% viability after 6 years in a desiccator.
- We examined 49 ionizing radiation sensitive strains of *D. radiodurans* and found that each was sensitive to desiccation.
- Chromosomal DNA from desiccated cultures revealed a time dependent increase in DNA damage, as measured by an increase in DNA double strand breaks.

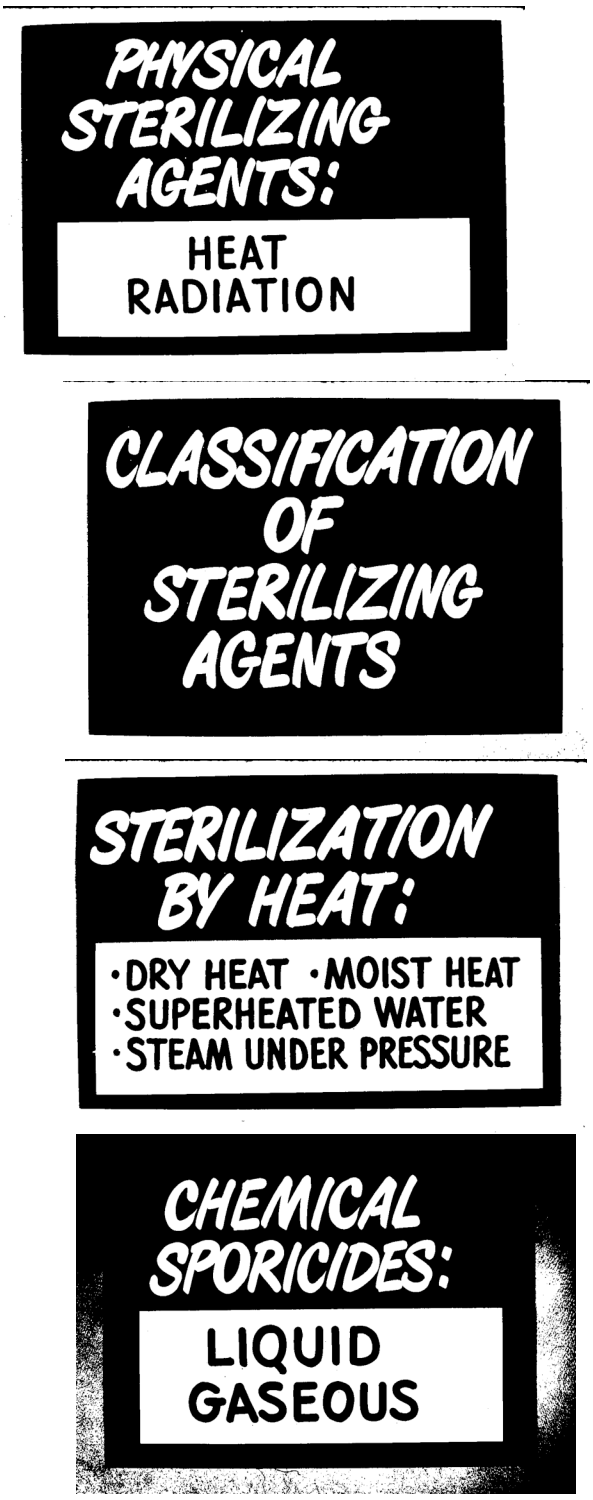
Table 1. Survival of 25 bacterial and one fungal species following one-week desiccation and to γ irradiation while desiccated.

Organism	Survival after 1 Week Desiccation*	Survival after 1 Week Desiccation and 2.5 Mrad γ Radiation
Escherichia coli K12	-	nd
Streptococcus fecalis	++	-
Streptococcus lactis	++	-
Streptococcus viridans	++	-
Staphylococcus aureus	++	-
Staphylococcus epidermidis	++	-
Bacillus subtilis	++	-
Bacillus megaterium	++	-
Bacillus sphaericus	++	-
Bacillus cererus	++	-
Serratia marcescens	-	nd
Mycobacterium smegmatis	++	-
Enterobacter aerogenes	++	-
Proteus vulgaris	-	nd
Alcaligenes faecalis	++	-
Sacchromyces cerevisiae	+	-
Psuedomonas aeruginosa	-	nd
Micrococcus luteus	+	-
Citrobacter freundii	-	nd
Deinococcus radiodurans R1	++	++
Geodermatophilus sp.	++	-
Cytophaga sp.	++	-
Rubrobacter radiotolerans	-	nd
Rubrobacter xylanophilus	-	nd
Acinetobacter radioresitens	+	-
Methylobacterium radiotolerans	++	-

*++, survival in excess of 1%; +, survival greater than 0.1% but less than 1%; -, below level of detection (less than 100 cfu/ml); nd, not determined

APPENDIX C4:
PLENARY PRESENTATION MATERIALS

Carl Bruch



Sterilization: process to destroy or remove all viable microorganisms from a product.
Terminal sterilization: validated process whereby product within its primary package is sterilized.

Sterilization (operational definition in 1950's):
Process by which viable microorganisms are killed or removed to the extent that they are no longer detectable in standardized culture media in which they previously have been found to proliferate.

Sterilization (definition considered by Planetary Quarantine Program in 1960s):
Process by which anticipated levels of microbial contaminants in a load of materials are exposed to that number of D-values for the sterilant being utilized so that the probability (calculated) for a survivor is ~ 10-6.

Biological Indicator (BI): calibrated population of microorganisms (of high resistance to the mode of sterilization being monitored) on or in a carrier within a package (which maintains integrity of inoculated carrier) that serves to demonstrate whether sterilization conditions were met; (2) sterilization process monitoring device consisting of a standardized viable population of micro-organisms (usually bacterial spores) known to be resistant to the mode of sterilization being monitored.

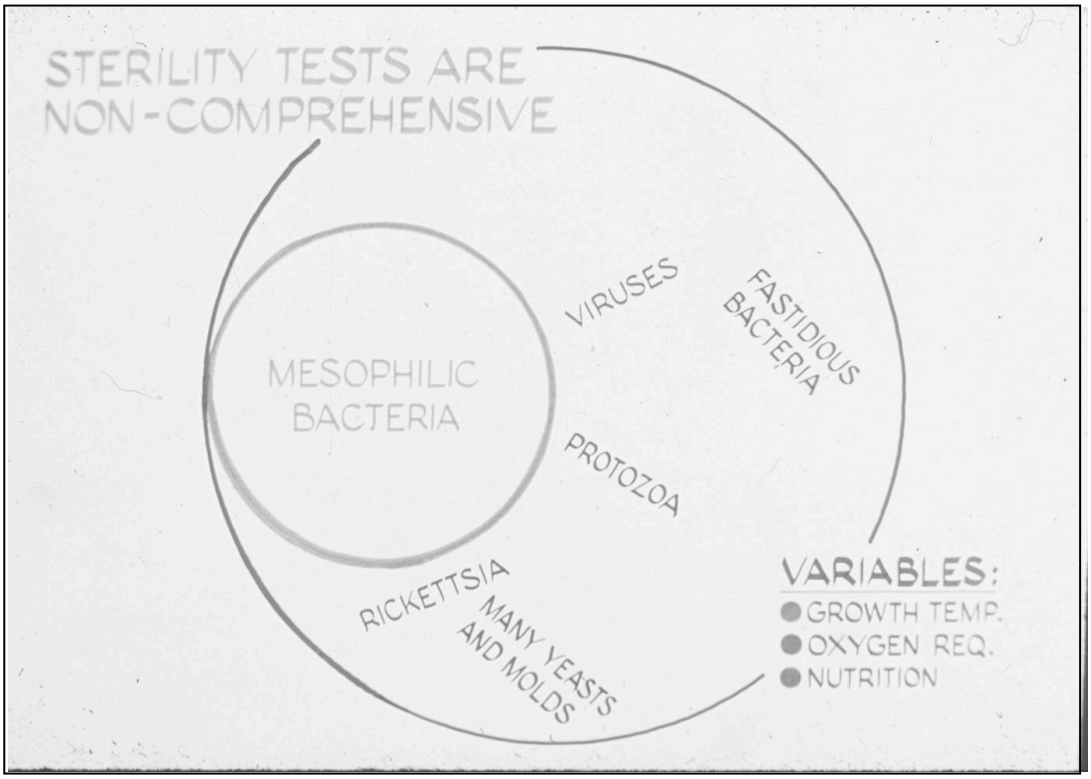
AAMI-TIR ON CHEMICAL STERILANTS
AND STERILIZATION METHODS
PUBLICATION DATE: JULY 1990

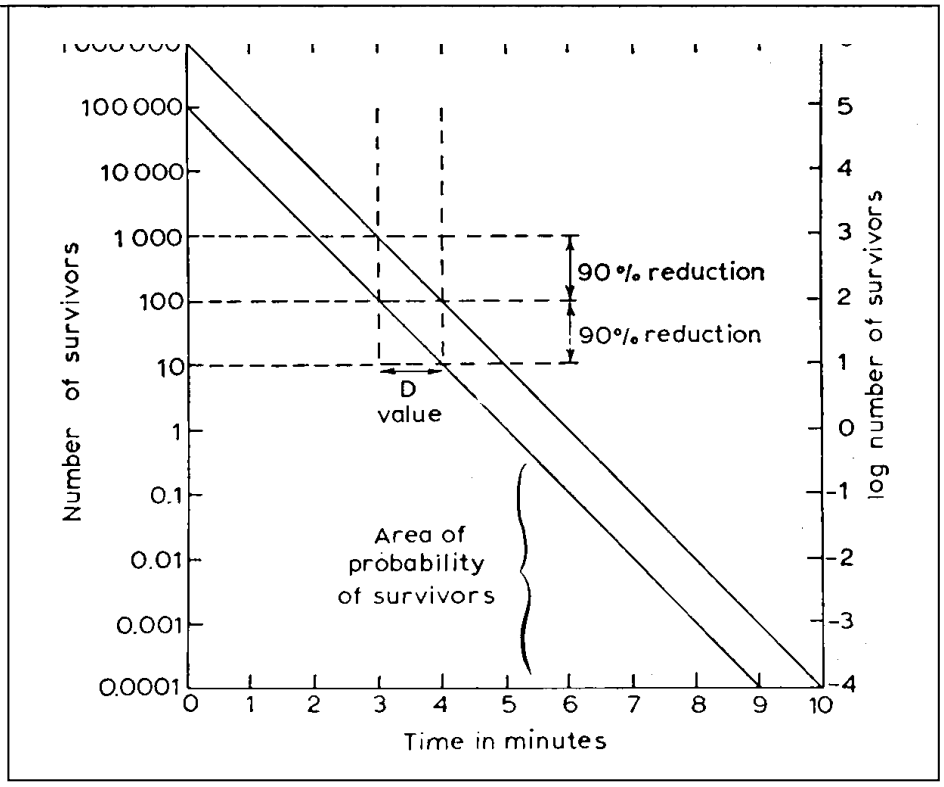
INTRODUCTION AND SCOPE
SAFETY (TOXICITY) AND PERFORMANCE CHARACTERISTICS

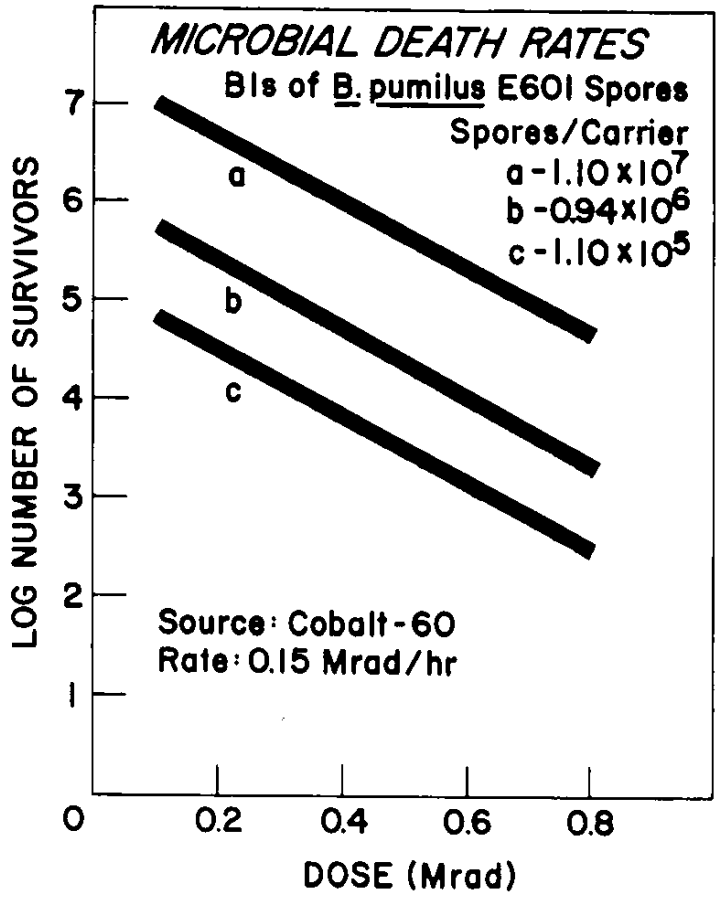
LIQUID STERILANTS	GASEOUS STERILANTS
GLUTARALDEHYDE	FORMALDEHYDE
PEROXYACETIC ACID/	CHLORINE DIOXIDE
HYDROGEN PEROXIDE	HYDROGEN PEROXIDE
SODIUM HYPOCHLORITE	OZONE

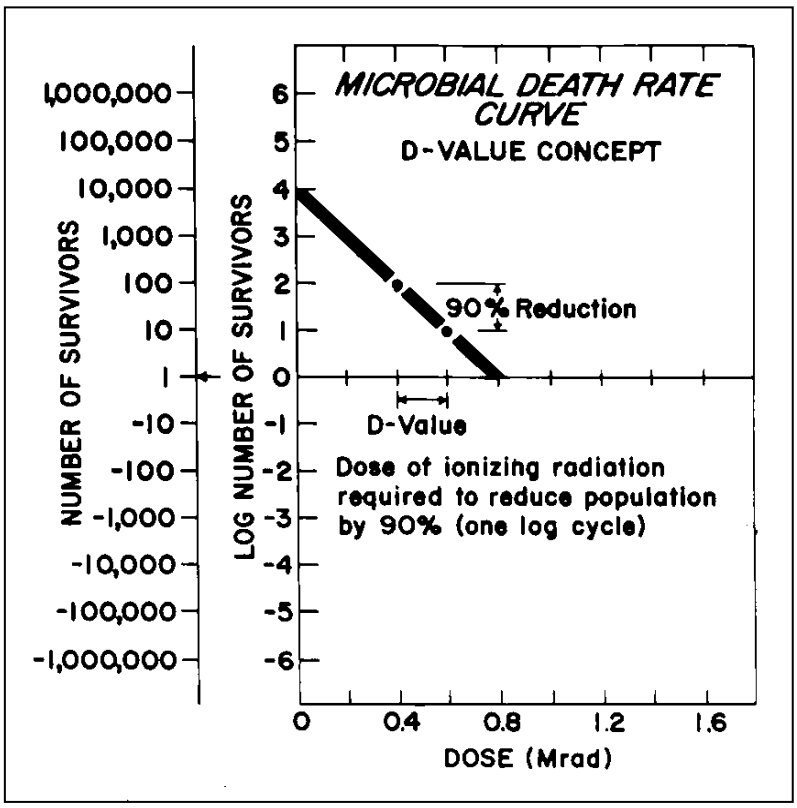
GOVERNMENT REGULATION (EPA, FDA, OSHA AND STATES)
SELECTION CRITERIA
GUIDELINES FOR USE
BIBLIOGRAPHY AND GLOSSARY

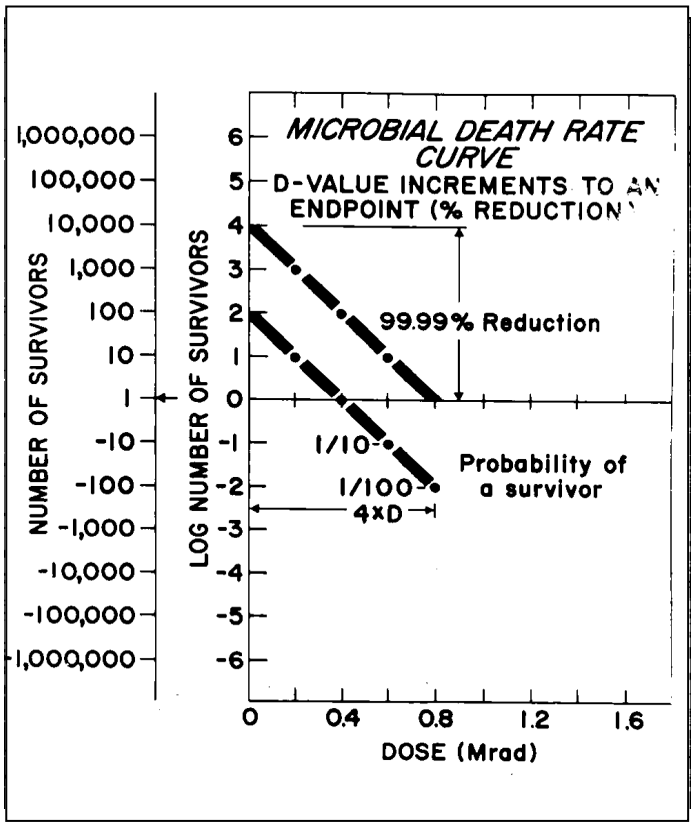
APPENDICES: MATERIALS COMPATIBILITY
MATERIAL SAFETY DATA SHEETS

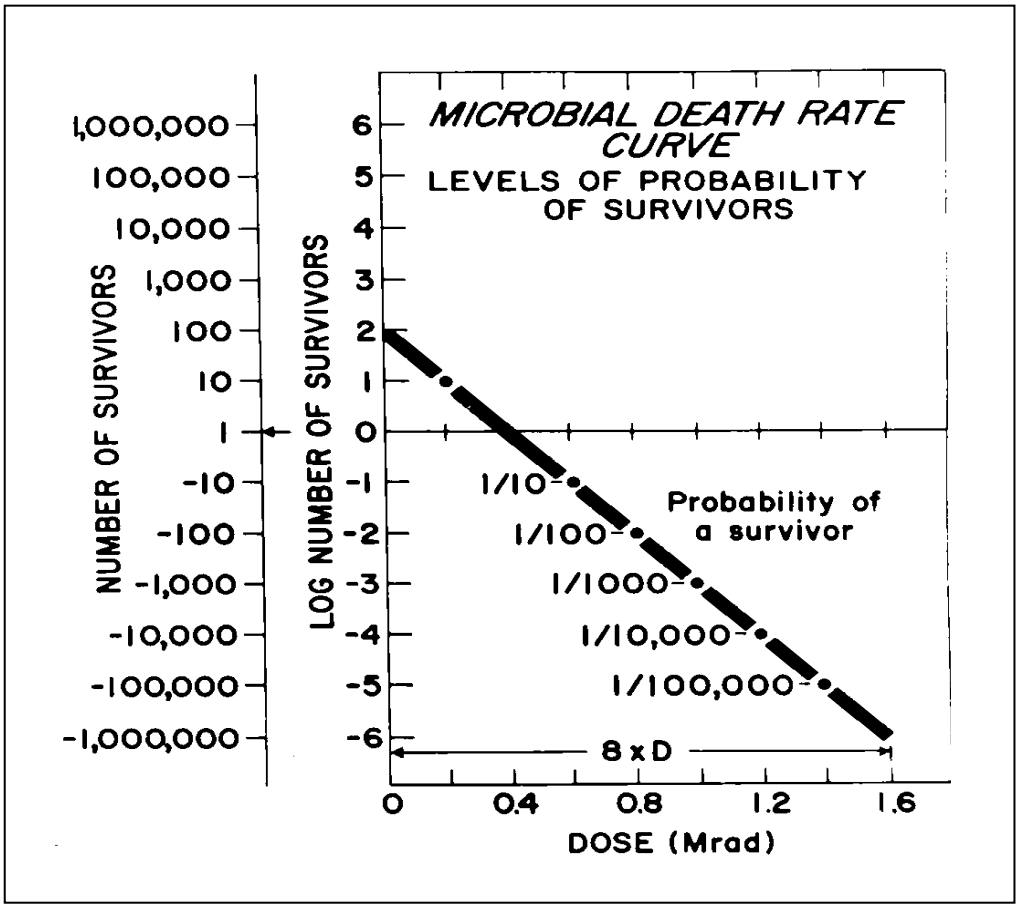



Number of survivors
log number of survivors
100 000
10 000
1 000
100
10
1
0.1
0.01
0.001
0.0001
5
4
3
2
1
0
-1
-2
-3
-4
90 % reduction
90% reduction
D
value
Area of
probability
of survivors
0 1 2 3 4 5 6 7 8 9 10
Time in minutes


MICROBIAL DEATH RATES
Bis of B. pumilus E601 Spores
Spores/Carrier
a - 1.10 x 10^7
b - 0.94 x 10^6
c - 1.10 x 10^5
a
b
c
Source: Cobalt-60
Rate: 0.15 Mrad/hr
LOG NUMBER OF SURVIVORS
7
6
5
4
3
2
1
0
0.2
0.4
0.6
0.8
DOSE (Mrad)


MICROBIAL DEATH RATE CURVE
D-VALUE CONCEPT
NUMBER OF SURVIVORS
1,000,000
100,000
10,000
1,000
100
10
1
-10
-100
-1,000
-10,000
-100,000
-1,000,000
LOG NUMBER OF SURVIVORS
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
90% Reduction
D-Value
Dose of ionizing radiation required to reduce population by 90% (one log cycle)
0
0.4
0.8
1.2
1.6
DOSE (Mrad)


MICROBIAL DEATH RATE CURVE
D-VALUE INCREMENTS TO AN ENDPOINT (% REDUCTION)
NUMBER OF SURVIVORS
1,000,000
100,000
10,000
1,000
100
10
1
-10
-100
-1,000
-10,000
-100,000
-1,000,000
LOG NUMBER OF SURVIVORS
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
99.99% Reduction
1/10
1/100
4xD
Probability of a survivor
0
0.4
0.8
1.2
1.6
DOSE (Mrad)



LOGARITHMIC DEATH

THE FOLLOWING EQUATION DESCRIBES ORDER OF DEATH:

$$K = 1/t \ (\log No - \log N)$$

WHERE K = A CONSTANT (ASSUMES LOGS TO BASE 10)

t = TIME OF EXPOSURE

No = NUMBER OF ORGANISMS VIABLE AT START OF TIME INTERVAL

N = NUMBER OF SURVIVORS AT END OF TIME INTERVAL

EXPRESSED IN NATURAL LOGARITHMS THE EQUATION IS:

$$N = N_o e^{-Kt}$$

DECIMAL REDUCTION VALUE

THE D VALUE IS DERIVED FROM THIS FORMULA WHEN A 90% REDUCTION OF POPULATION OCCURS:

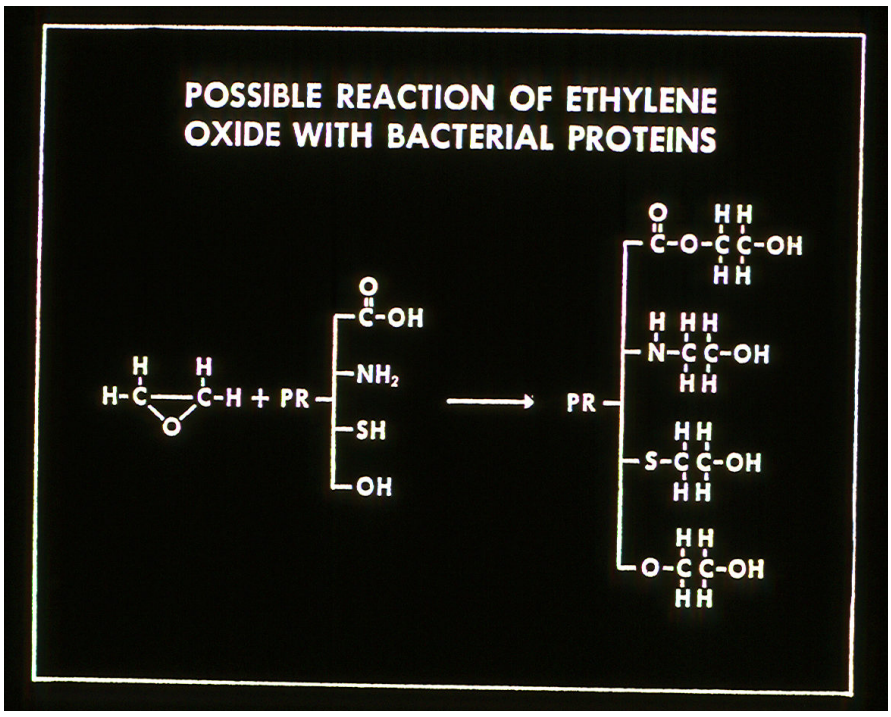
$$K = 1/t \ (\log No - \log 0.1N_o)$$

$$K = 1/t \ (1) = 1/t$$

$$t = 1/K$$

TIME t IS DEFINED AS THE DECIMAL REDUCTION TIME:

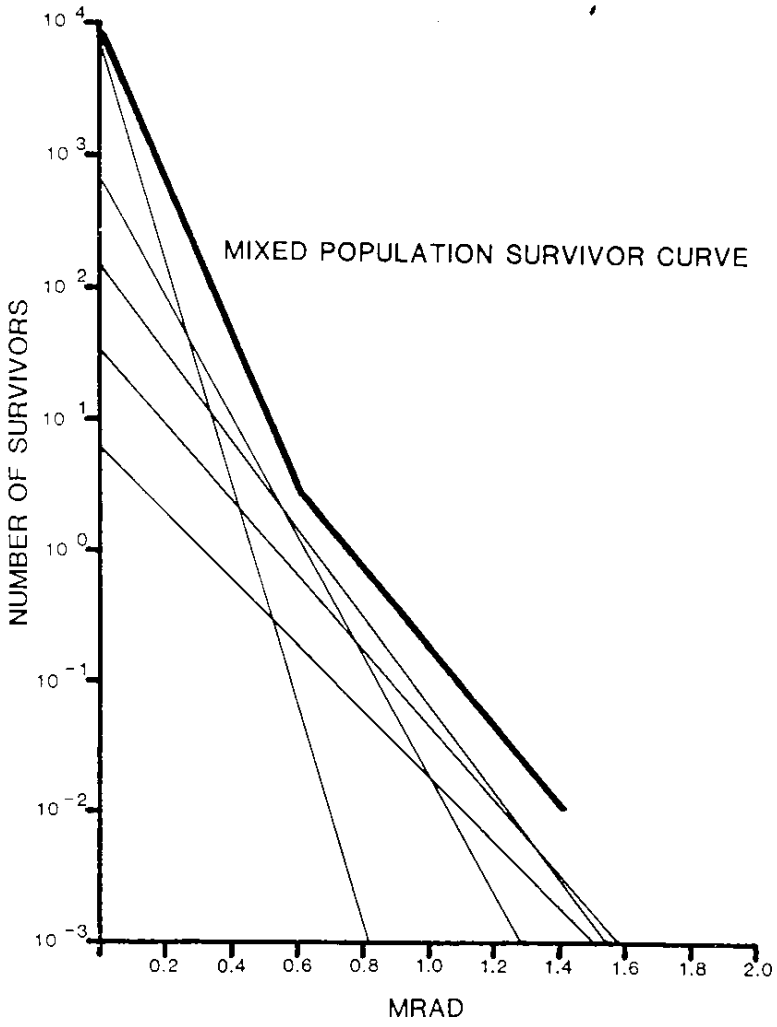
$$D = 1/K$$

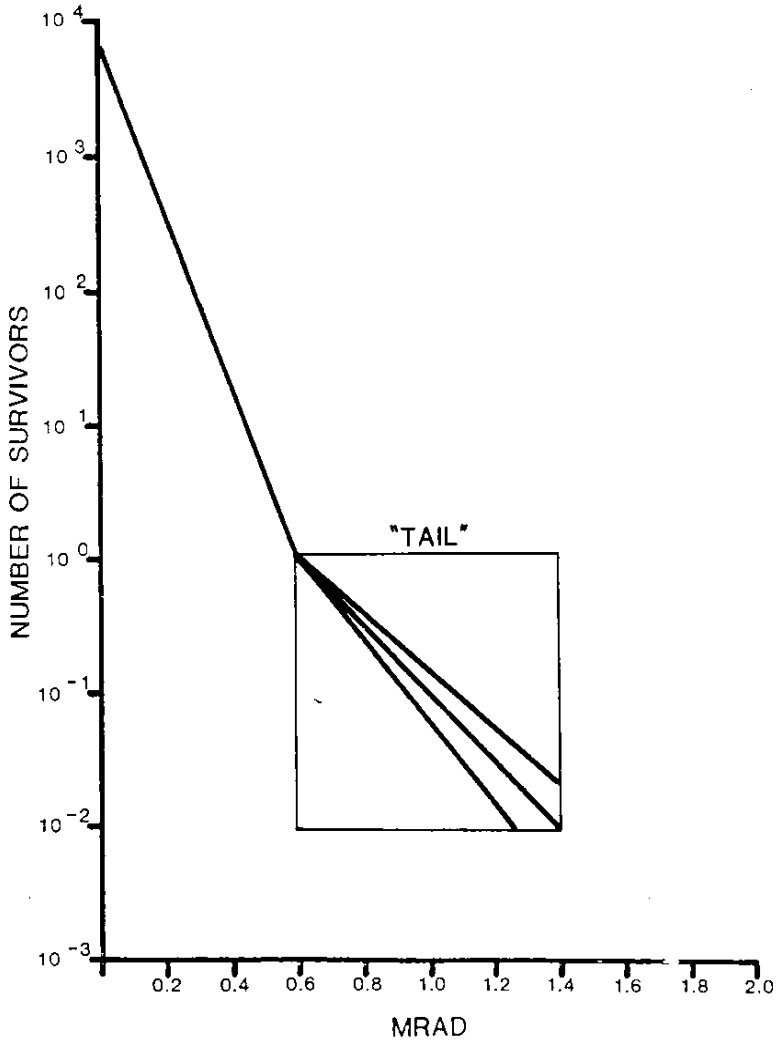


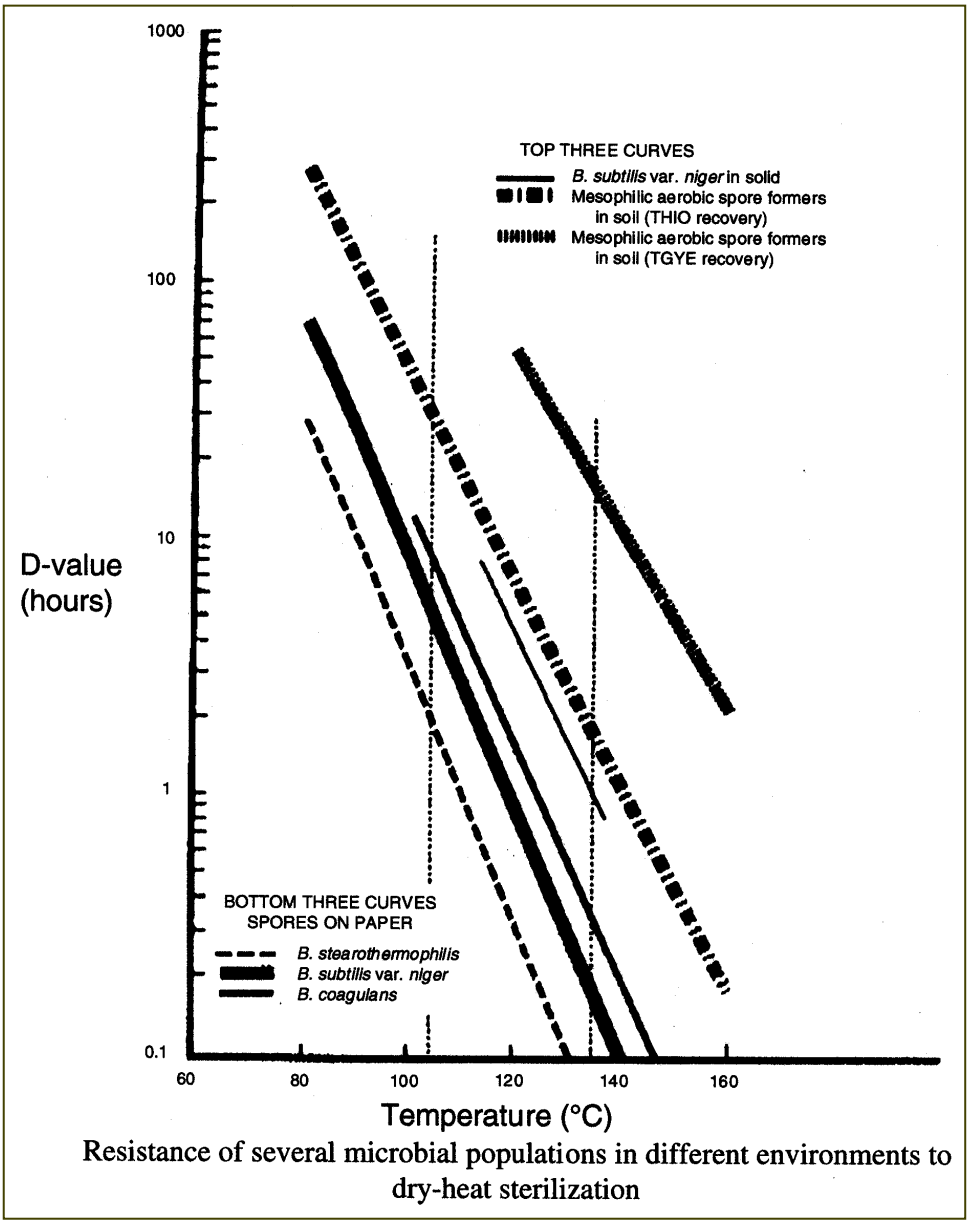
SOME CHEMICAL PROCESSES FROM RADIATION OF PLASTICS

1. CROSSLINKING
   - RADIOLYSIS
   - HYDROGEN ABSTRACTION
   - COMBINATION OF POLYMERIC RADICALS

2. DEGRADATION
   - SCISSION OF MOLECULAR CHAIN
   - GAS EVOLUTION (H_2, CH_4, HCl, ETC.)
   - OXIDATIVE DEGRADATION
     - (HYDROPEROXIDES)


MIXED POPULATION SURVIVOR CURVE
NUMBER OF SURVIVORS
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
MRAD


NUMBER OF SURVIVORS
10 4
10 3
10 2
10 1
10 0
10 -1
10 -2
10 -3
"TAIL"
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
MRAD



Resistance of several microbial populations in different environments to dry-heat sterilization

Table 2. D Values for Sterilization by Hot Air*

Temperature (°C)	D Value (Hours)
105	19
110	12
115	7
120	4.5
125	2.7
130	1.7
135	1.0
140	0.62
145	0.38
150	0.23 (13.8 minutes)
155	0.14 (8.4 minutes)
160	0.087 (5.2 minutes)

*Data taken from Bruch (10); these values represent the resistance of dry (washed) spores of *B. subtilis* var. *niger* trapped in dental plastics. The resistance of these spores dried on paper or glass would be approximately 50% of these values.

Table 3. D Values for Radiation Sterilization*

Organism	D Value Mrads
Coxackie virus A-9	0.4 – 0.65
Streptococcus faecium	0.3 – 0.4
Micrococcus radiodurans	0.3 – 0.45
Bacilllus subtilis var *niger* (spores)	0.1 – 0.15
Bacillus pumilis (spores)	0.15 – 0.25
Bacillus stearothermophilus (spores)	0.05 – 0.1
Colstridium sporogenes (spores)	0.2 – 0.3
Clostridium botulinum (spores)	0.25 – 0.35
Aspergillus niger (spores)	0.05

*See papers in reference (14) and also Silverman and Sinskey (40) for further information on microbial resistance to ionizing radiation.

APPENDIX C5:
PLENARY PRESENTATION MATERIALS
Martin Favero

PLANETARY QUARANTINE
Spacecraft Sterilization and Sample Return Issues

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NASA WORK SHOP

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OVERVIEW-1

- **Sterilization Principles**
  - **Current Methods**
  - **Requirements**
- **Cleaning Principles**
  - **Current Methods**
  - **Requirements**
- **Relationship to Sterilization**

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OVERVIEW-2

Viking Experience

- First approaches
  - Make and bake
  - Use of BI B. Subtilus var. niger
- Naturally occurring bioburden
  - D values of spores associated with S/C
  - Super Spore
  - Ribbon Experiments

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OVERVIEW-3

- **Lunar Receiving Laboratory**
  - **-Sample sterilization**
  - **-Rock Box decontamination**
  - **-Issues**
- **Mars Sample Receiving Laboratory**
  - **-Sample sterilization**
  - **-Sterilization issues**

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Sterilization Methods

Heat

Gas

Radiation

Liquid Chemical

New low Temperature Sterilization Systems

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STERILIZATION

A PROCESS THAT KILLS ALL MICROORGANISMS

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STERILITY

COMPLETE ABSENCE OF LIVING MICROORGANISMS

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Operational Definition of Sterilization

A carefully designed and monitored process that will assure the probability of an item being contaminated to be equal to or less than one in one million (SAL = 10^{-6})

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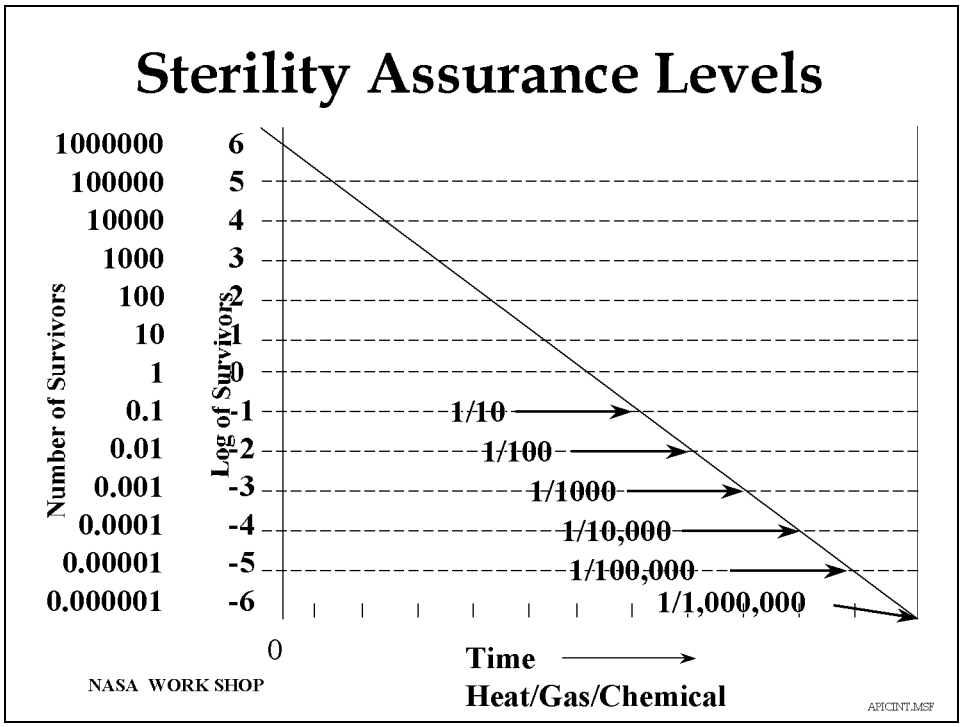

FOOD INDUSTRY

- Safety Assurance Level (SAL)
- Based on inactivation of *Clostridium botulinum* spores
- Food Processing Cycle = 12 D Values
- SAL = 10^{-11}

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MEDICAL DEVICES AND SAL'S

- 1965 Sweden Public Health Required a SAL of 10^{-6} Medical Devices Labeled Sterile - Lars Kallings
- In the 1970's FDA Incorporated SAL's into FDA Regulations; Carl Bruch Link to NASA and FDA
- 1979 Canada Proposed Two SAL's, 10^{-3} and 10^{-6} Based on End Use or Product Tolerance

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Estimates of Probability of Survivor for Sterilized Items[1]

Items	Probability of Survivor/Unit
Canned chicken soup	10^{-11}
Large volume parenteral fluid	10^{-9}
IV catheter and delivery set	10^{-6}
Syringe and needle	10^{-6}
Surgical drape	10^{-3} to -6
Urinary catheter	10^{-3}
Endoscope processed with liquid chemical germicide	? to 10^{-3}

1) The USP 20 item sterility test will detect probability of surviv or/unit of $10^{-1.3}$ with 95% confidence

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PLANETARY QUARANTINE

- **Spacecraft Sterilization Accomplished by Dry Heat**
- **Probability of Landing a Contaminated Spacecraft on Mars=10^{-4} per Mission**

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SAL DEFINITIONS

- **1/1,000,000 survival of challenge of 10^6 resistant spores**
- **1/1,000,000 survival of bioburden on load - sample**
- **1/1,000,000 chance than there will be an adverse reaction - humans, plants, environment**

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SAL OF 10^{-6}

- **Starting with 10^6 resistant spores the sterilization procedure results in 10^{-6} probability that one spore survives**
- **A 10^{-6} probability that a contaminant will survive on a product after sterilization**
- **Less than one non-sterile in 10^6 items**

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SAL DEFINITIONS - 2

- **1/1,000,000 chance one viable organism will contaminate planet**
- **1/1,000,000 chance one viable organism will grow on planet**

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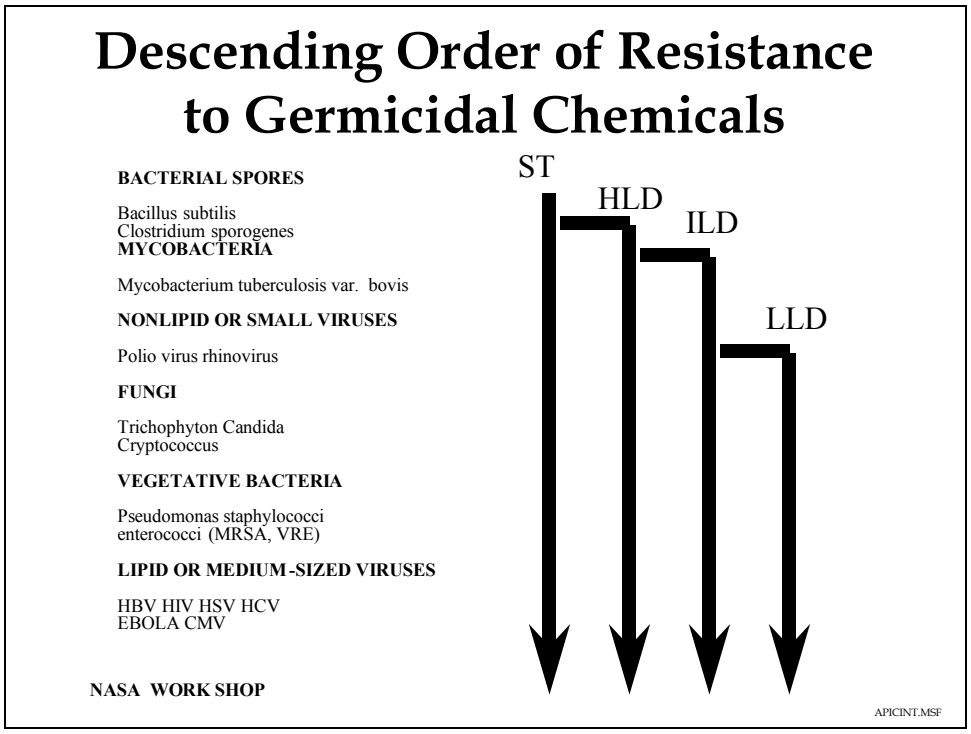

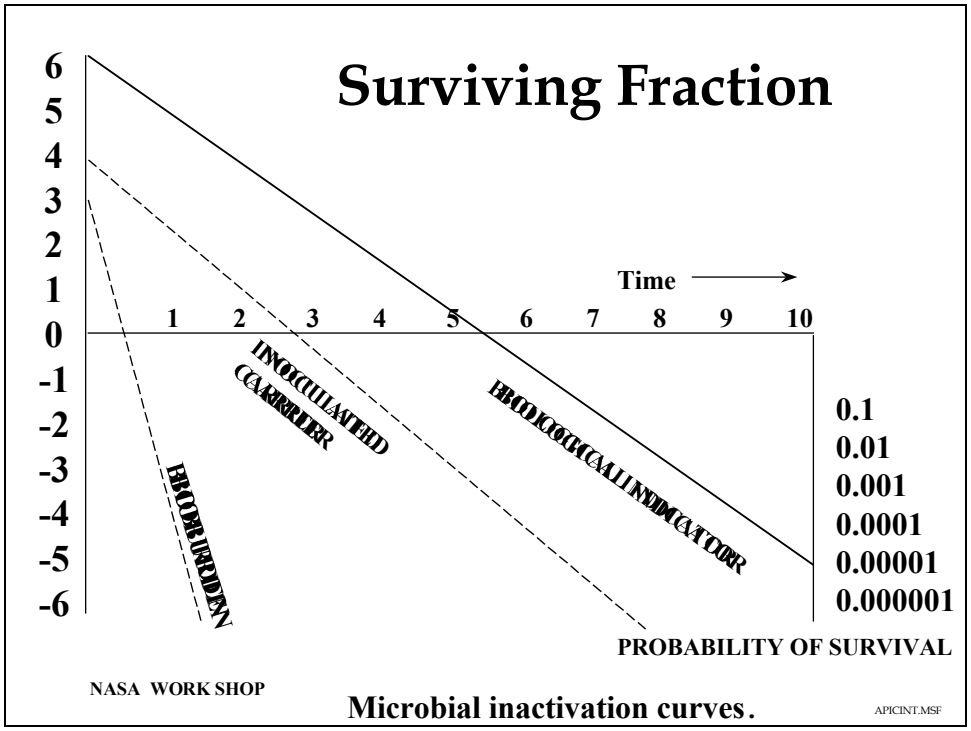

Microbial inactivation curves.

THE FUTURE FOR STERILIZATION

- **Lower Technology → Higher Technology**
- **Faster Sterilization Times**
- **Lower Temperatures**
- **Better Material Compatibility**

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NEW EMERGING TECHNOLOGIES

- **Hydrogen Peroxide Gas Plasma STERRAD/J&J**
- **Chlorine Dioxide Gas ISODEX J&J**
- **Hydrogen Peroxide Vapor STERIS/AMSCO**

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VIKING

- **Sterilized by dry heat 113C for 18-24 hrs; parts and systems prequalified at 135 C; cycle based on bioburden determination and lethality correction**
- **Purpose**
  - **prevent contamination of life detection systems**
  - **prevent contamination of the planet**

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SPACECRAFT STERILIZATION

- First approaches
  - Make and bake
  - Use of BI B. Subtilus var. niger
- Naturally occurring bioburden
  - D values of spores associated with S/C
  - Ribbon Experiments
  - Super Spore

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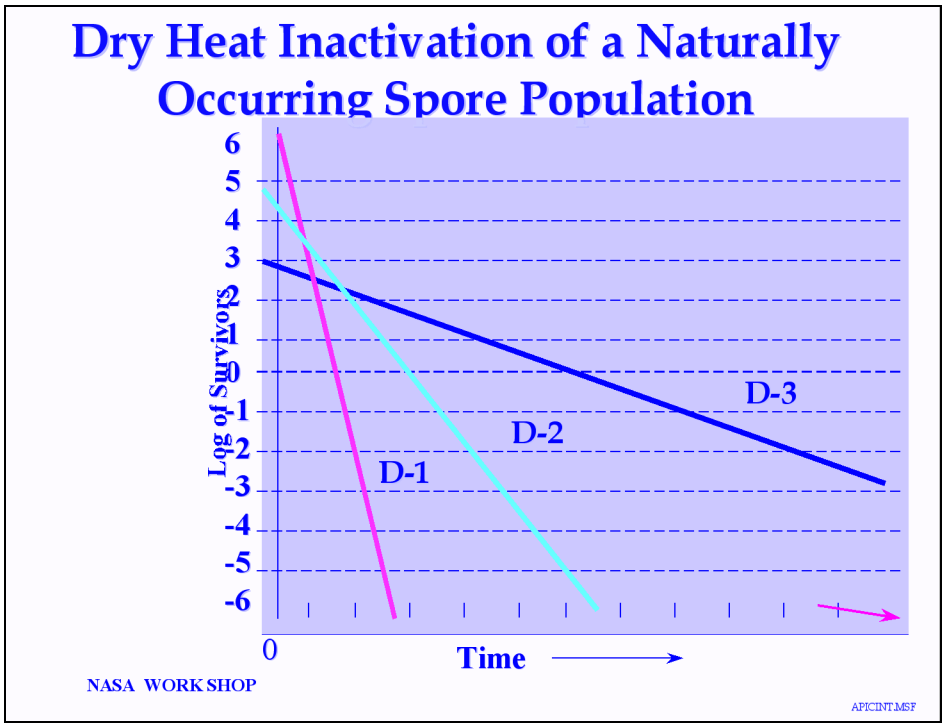
Dry Heat Inactivation of a Naturally Occurring Spore Population
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
Log of Survivors
D-1
D-2
D-3
0
Time
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TEFLON RIBBON EXPERIMENTS CYCLE VERIFICATION – 1

Experiment Number	Temp. Cycle	N_O Spores	Positive / Total	MPN
2	113	1.4 x 10	1/24	0.042
5	113	4.2 x 10	1/22	0.047
6	113	4.2 x 10	6/24	0.287
7	113	5.3 x 10	1/12 T	0.087
			1/12 T/F	0.087
8	113	5.8 x 10	1/12 T	0.087
			2/12 T/F	0.182
9	113	3.5 x 10	3/12 T	0.287
			1/12 T/F	0.087

R.H. = 0.133% (1.2 mg/L or 1500 ppm water)

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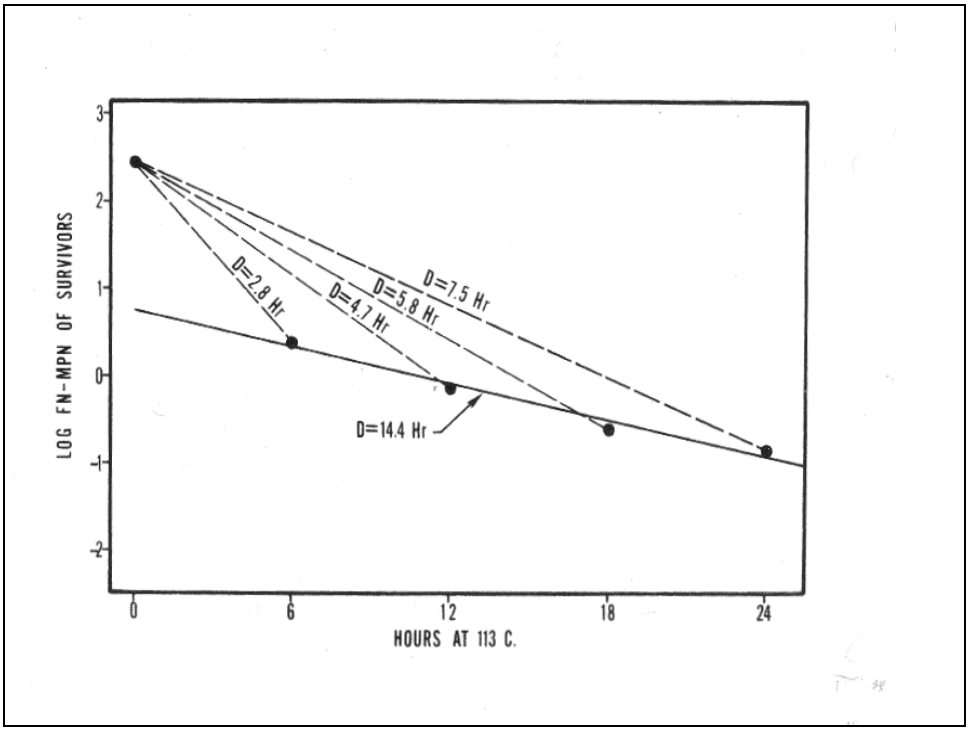

APOLLO 11-12

- Returned samples placed in quarantine
- Purpose
  - to prevent exposure of lunar material to humans, animals, plants and the environment
  - to protect the lunar material from terrestrial biological and chemical contamination

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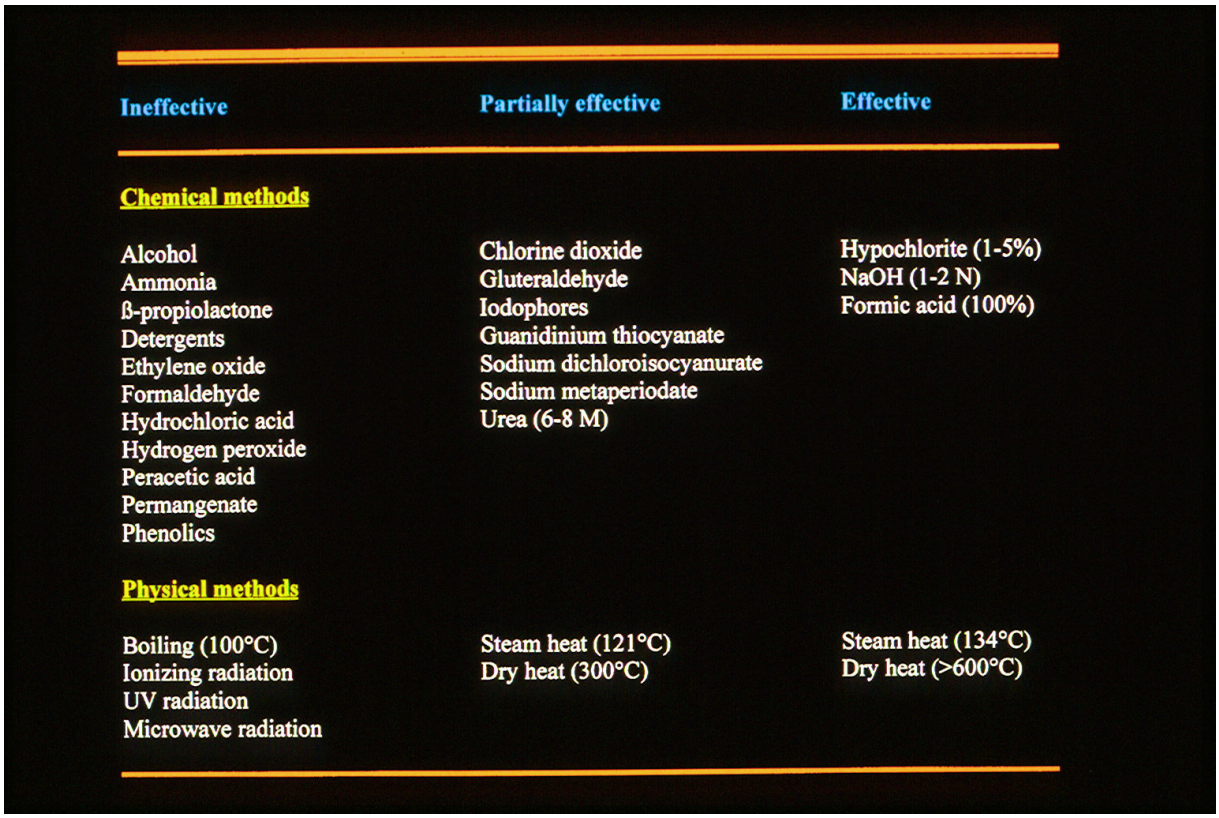
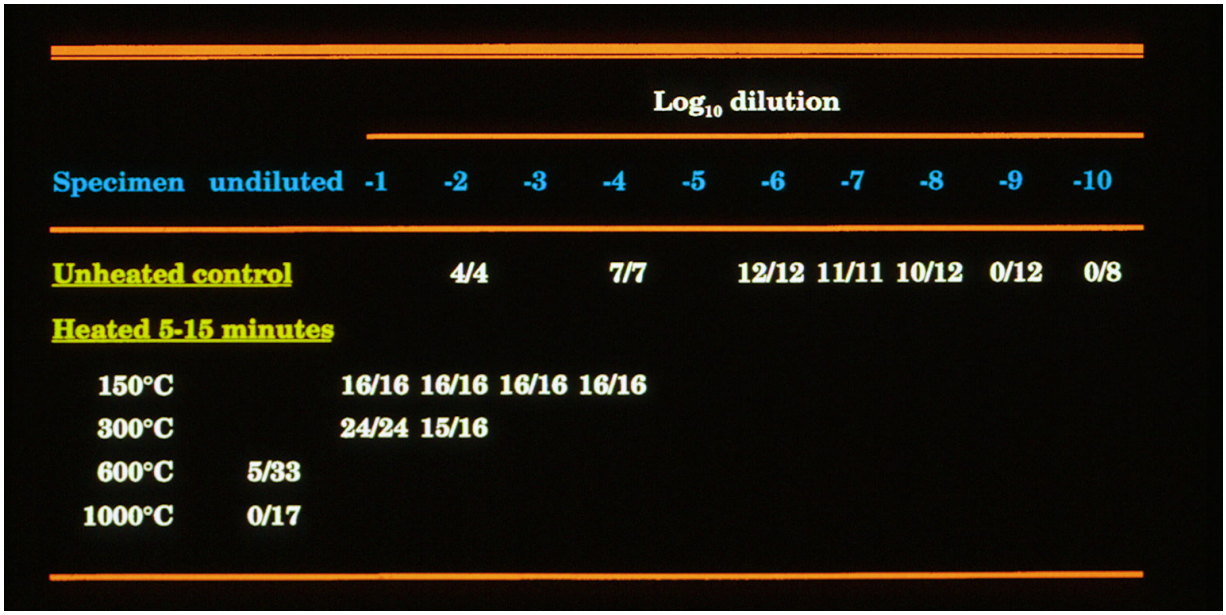
APPENDIX C6:
PLENARY PRESENTATION MATERIALS
Paul Brown



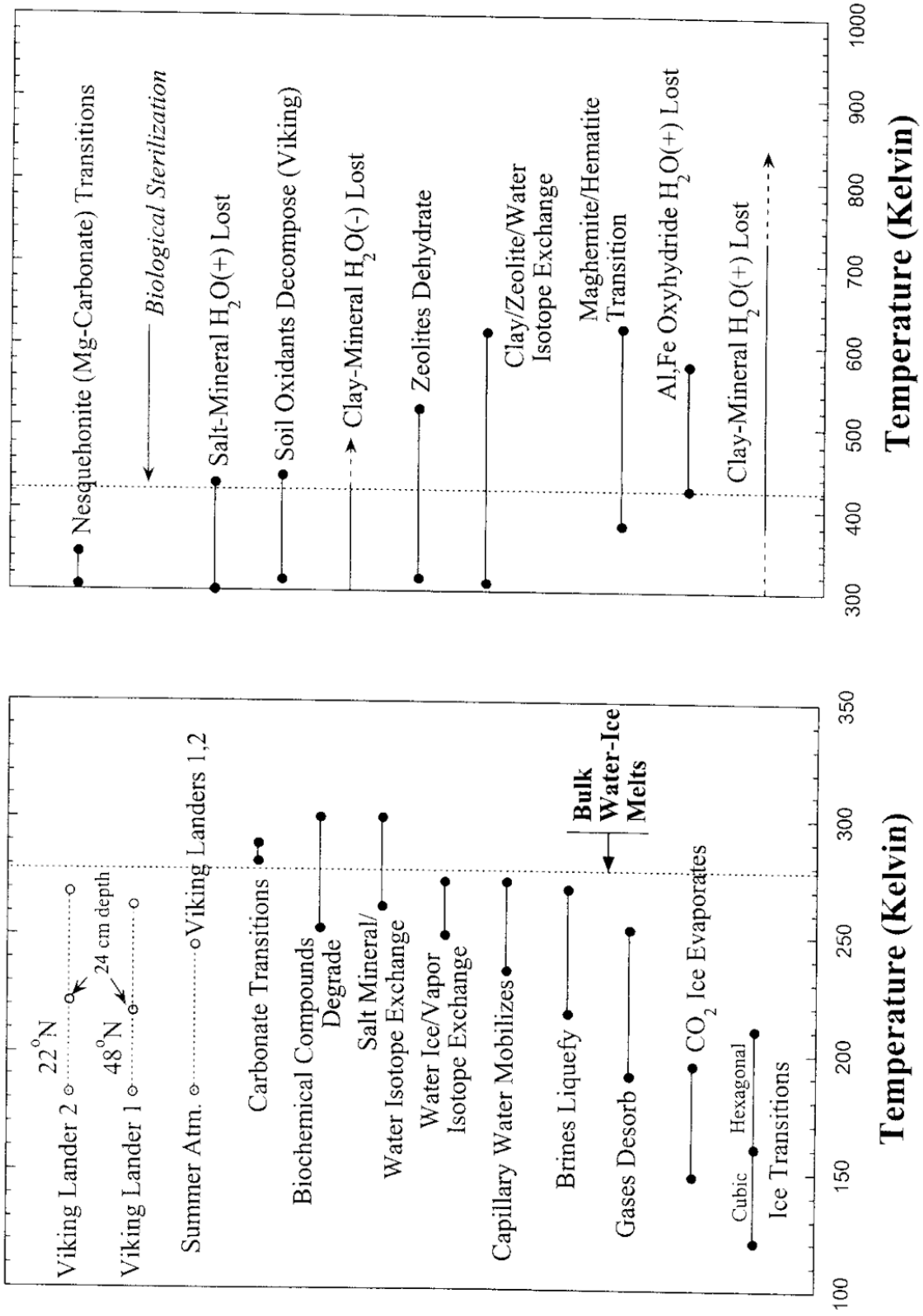
Ineffective	Partially effective	Effective
Chemical methods		
Alcohol	Chlorine dioxide	Hypochlorite (1-5%)
Ammonia	Gluteraldehyde	NaOH (1-2 N)
ß-propiolactone	Iodophores	Formic acid (100%)
Detergents	Guanidinium thiocyanate	
Ethylene oxide	Sodium dichloroisocyanurate	
Formaldehyde	Sodium metaperiodate	
Hydrochloric acid	Urea (6-8 M)	
Hydrogen peroxide		
Peracetic acid		
Permangenate		
Phenolics		
Physical methods		
Boiling (100°C)	Steam heat (121°C)	Steam heat (134°C)
Ionizing radiation	Dry heat (300°C)	Dry heat (>600°C)
UV radiation		
Microwave radiation		



	Log_{10} dilution									
Specimen	undiluted	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
Unheated control			4/4		7/7		12/12	11/11	10/12	0/12	0/8
Heated 5-15 minutes											
150°C			16/16	16/16	16/16	16/16					
300°C			24/24	15/16							
600°C	5/33										
1000°C	0/17										

Treatment	Inactivation temperature or final concentration of chemical (wt/vol)		Decrease in log LD_{50} after exposure for			
			15 min	60 min	15 min	60 min
			CJD		Scrapie	
Untreated		-	4.8-6.2		9.3-10.0	
Steam autoclave		121°C	≤4.0	5.0		≥8.3
		132°C	≤4.0	≥5.5		≥8.8
NaOH	pH 9.9	0.01 N		1.0	0	1.0
	12.5	0.1 N	4.8	4.8	5.0	6.0
	13.3	1.0 N	≤3.0	≥5.0	6.0	≥6.8
NaOCl		0.5%			4.5	4.5
		1.0%			6.0	6.0
		2.5%		3.3	≥6.0	≥7.0
$KMnO_4$		0.1%		0	0.5	1.2
		0.4%			1.0	2.0
		0.8%		2.3		
Iodine		2%				2.1
Lysol (alcoholic phenol)		10%				1.5
Urea		4.0 M			0	≤1.0
		8.0 M		≤0.8		0
NH_3		1.0 M				1.0
HCl	pH 0.5	1.0 N		≤1.8		
Formaldehyde		3.7%		0		0.8
Gluteraldehyde		5-12.5%	incomplete		incomplete	
Ethanol		100%				0
Organic solvents						
Acetone		100%				2.5
Chloroform-methanol		100%(2:1)				1.1-1.6
n-butanol		10%				0.2-0.7
Paraffin-embedded tissue blocks: still infective after years of storage						

APPENDIX C7:
PLENARY PRESENTATION MATERIALS
(Source Unknown



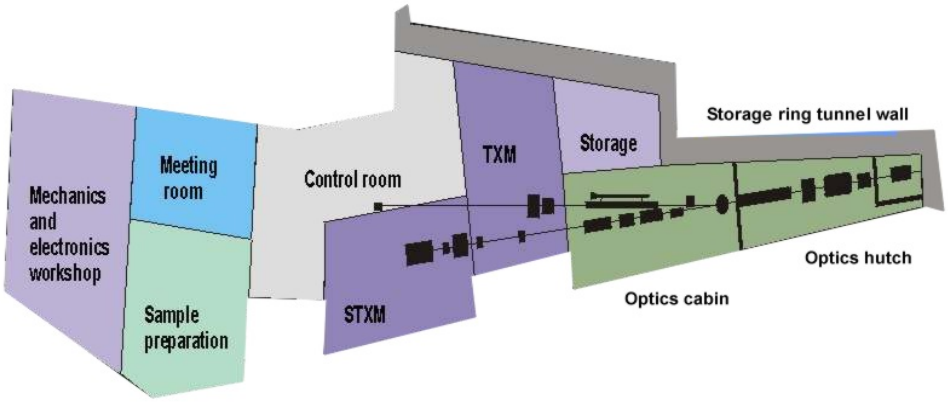
APPENDIX D:
SCANNING X-RAY MICROSCOPY

(Supplement to Sub-group 1B)

Introduction:

Third generation synchrotron sources produce a beam of unprecedented quality: the extremely low emittance coupled with high brilliance together with the versatility of new insertion devices, offer the capability to control brightness, spectrum and polarisation, coherence and size of the beam. This means that X-ray microscopy techniques which have been intensively used in the soft X-ray region (see "Other X-ray microscopy groups") can now be extended, with the anticipation of very high performance to higher photon energies. This will enable new investigations: study of thicker specimens, access to K absorption edges of elements of major interest in the biological and materials sciences, in particular from Potassium to Chromium, access to M and L edges of heavy metals (i.e, Au or Ag) for specimen labeling, and the use of X-ray fluorescence for trace element mapping.

The ID21 X-ray microscopy beamline at the ESRF houses two branchlines: one is dedicated to scanning microscopy techniques (SXM) and the second to full-field imaging microscopy (TXM).



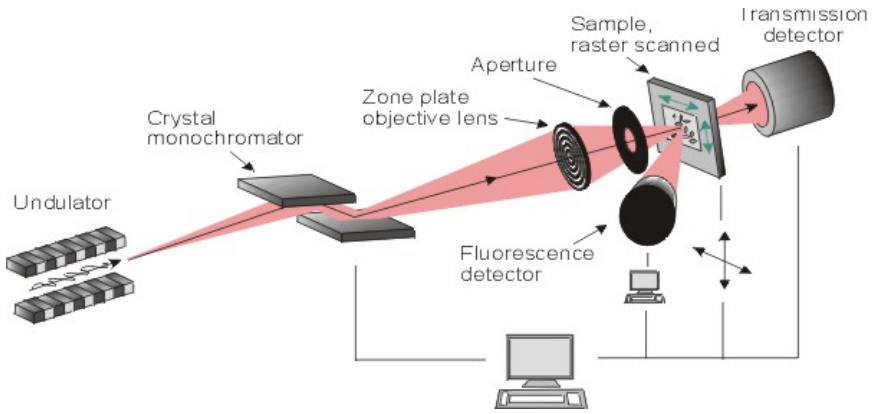
The SXM is designed for use over a relatively wide spectral range from 0.2 - 8 keV giving access to absorption edges from a wide range of elements of interest in life, materials and environmental sciences. The microscope is designed to accept, apart from conventional absorption contrast imaging, a variety of complementary imaging modes, in particular spectromicroscopy using both fluorescence imaging and scanning of the primary X-ray probe energy for XANES imaging. The STXM itself works comparably to the different known scanning microscopes: The source - in this case the storage ring radiation emitted by the insertion device - is demagnified by a zone plate into a focal spot with a diameter below 1µm. In the focal spot plane, the object is aligned and is scanned across the focal spot. The different signals from interaction of X-rays with matter like fluorescence, absorption or XAS can be detected with high spatial resolution.

The TXM, designed to work in the photon energy range of 2.5 - 8 keV, works analogously to an visible light microscope: The beam is condensed onto a sample and a high spatial resolution objective magnifies the image into a spatial resolving detector. The short exposure times are essential for X-ray tomography.This microscope has been designed to take a full image of the sample in one shot in the second or minute range at very high spatial resolutions below 100nm. Next to absorption contrast, the TXM offers the possibility of spectromicroscopy for element mapping and XANES.

The beamline is a windowless UHV beamline - continuous with the storage ring vacuum - and therefore necessitates the use of UHV technology throughout. The white beam from the undulator source is conditioned in the lead shielded optics hutch principally by means of a fixed exit double mirror system acting as a low band pass filter. The glancing angle of this device can be varied from 7-20 mrad using either Pt, Si or Ni reflective coatings, thus allowing harmonic rejection factors greater than 1e-3 for any energy between 1-10 keV for spectroscopic experiments for total transmissions greater than 70%. This system reduces the high energy content of the beam sufficiently to eliminate the need for lead shielding in the subsequent cabin. The optics cabin houses a multilayer carousel system which deflects part or all of the beam to pass down the side branch - simultaneously acting as a pre-monochromator. This system potentially allows both branches to be used simultaneously - the direct one for a STXM, the side branch for a TXM. The Optics cabin also houses a series of pre-focussing optics for the direct branch for use in combination with its monochromators as well as the monochromating and condensing optics for the side branch. The beam passes afterwards in the experimental cabins for the STXM or the TXM. For a detailed description use the imagemap.

General description of the ID21 scanning X-ray microscope:

The STXM is conceived to work with zone plate type optics that currently offers the proven best performance for this type of application. Clearly a single zone plate will be incapable of providing efficient focusing over the entire spectral range available and one of the challenging aspects of the microscope design has been to facilitate the remote exchange of the zone plate allowing close to optimum focusing conditions regardless of the operating energy. A small pinhole aperture (50µm to 50µm) will be used as a secondary source, set at about 1m from the zone-plate. This aperture and the microscope are mechanically linked to the same support in order to minimize relative movements (mechanical vibrations) between the two components. Even in an experiment requiring spatial coherence, the optimum amount of phase-space to accept is rather more than a single mode and involves a trade-off of high coherence - for diffraction limited focusing - against flux - which is required for observation of weak contrast phenomena and fluorescence experiments. Moreover, an overfilling of the aperture used as secondary source and the zone-plates allows the effects of beam instability to be minimized. The aperture size and the distance between the pinhole and the microscope are variable to accommodate a wide variety of microscopy, spectro-microscopy and related coherence experiments over a large energy range.



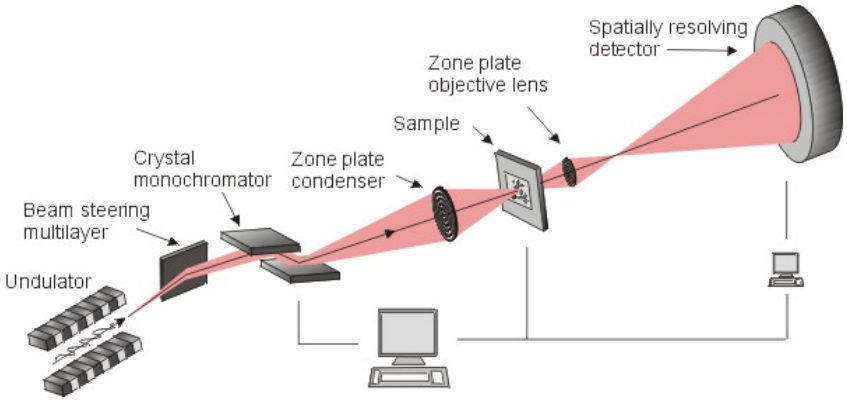
The X-ray microscope will be placed in an environmental chamber allowing operation in air, helium or vacuum (10^{-4} - 10^{-6} mbar). The entire microscope can be moved along the beam axis relative to the fixed exit pinhole which acts as the secondary source. This movement allows the pinhole-zone plate distance to be varied from 0.3 to 1.5m and allows the illumination conditions of the zone plate to be adapted to the experiment. Taking into account the mirror reflectivities, monochromator band pass and undulator characteristics the estimated photon flux in a 50nm probe is of the order of 10^9 - 10^7 ph/s. The wide spectral operating range of the microscope is attractive for spectro-microscopy. While

in its simplest form this might consist of taking multiple images of a single sample region at different incident energies, an interesting extension is to perform highly spatially resolved XAS scans on small regions of the sample. The spatial resolution of this mode is potentially limited by the probe size, convergence and the sample thickness, but requires careful mechanical design due to the energy dependence of the zone plate focal length. The sample will be scanned using a combination of piezo driven flexure and mechanical stages giving a total scan area of 10x10mm^2. The current aim is to approach pixel rates of 1kHz.

A manual sample rotation will be available, primarily for fluorescence mode imaging, but with the possibility of future upgrade to motorized movement for micro-tomography measurements The microscope design is intended to offer maximum flexibility for the use of various different detector types. Currently it is planned for absorption measurements to use alternatively, proportional gas detectors, PIN photodiodes and avalanche photodiodes. A high-energy resolution Germanium solid state detector will also be available for fluorescence measurements. A kinematically mounted sample holder has been developed. This should allow regions of interest to be identified and recorded on a standard light microscope prior to transfer into the X-ray microscope and rapidly aligned to the probe scan. It is intended for the same holder to be used on the full field imaging microscope allowing rapid transfer between the two endstations. The microscope will be controlled using essentially standard ESRF VME based electronics running OS9 with a user interface running on Unix workstations.

General description of the ID21 TXM:

The transmission X-ray microscope (TXM) or full field imaging microscope is basically conceived to work in Zernike phase contrast but the design of the microscope is intended to offer maximum flexibility for the use of various contrast modes. Zone plate type optics are used which currently offer the best proven performance for this type of application. The microfocussing objective zone plate, the condenser zone plate are variable to accommodate a wide variety of microscopy, spectromicroscopy and related experiments over an energy range of 3-6 keV. Spectromicroscopic applications are difficult to be used in fluorescence mode, this microscope offers therefore the full capability to use XAS in transmission.



The beam deflected by the multilayer assembly is monochromatized by a shaped fixed exit channelcut monochromator offering an energy resolution of 5.10-4. Alternatively, the multilayer can be used as monochromator. The beam is downstream focussed into the sample by a condenser zone plate. Two rotating mirror between the condenser optic and the sample adapt the numerical aperture of the beam and generate a cone illumination in order to avoid zero order light in the image plane. The light diffracted by the sample is afterwards focussed by a micro zone plate. The up to 1000x -

2000x magnified image is detected by a backside illuminated CCD coupled with an optical phosphor or scintillator system. Other detectors are envisaged and in discussion.

A kinematically mounted sample holder is in development for samples of up to a few mm. This should allow regions of interest to be identified and recorded on a standard light microscope prior to transfer into the X-ray microscope and rapidly aligned to the probe imaging. It is intended for the same holder to be used on the scanning transmission X-ray microscope allowing rapid transfer between the two endstations.

A sample rotation is foreseen and in construction for tomography mode imaging. Furthermore a cryogenic sample stage will be available allowing imaging the sample under liquid nitrogen conditions.

The microscope is controlled using essentially standard ESRF VME based electronics running OS9 with a user interface running on Unix workstations. A suited fast image processing software will be developed.

APPENDIX E: REFERENCES

Allen, C. C., et al. (2000): Effects of sterilizing doses of gamma radiation on Mars analog rocks and minerals. *J. Geophysical Research* **104**, 27043-27066.

Piccirilli, J. A., T. Krauch, S. E. Moroney, S. A. Benner (1990): Extending the genetic alphabet. enzymatic incorporation of a new base pair into DNA and RNA. *Nature* **343**, 33-37.

Pollard, E. C. (1953): *The Physics of Viruses*. Academic Press, NY, p. 53.

Race, Margaret S. and John D. Rummel, Editors (2000): Mars Sample Handling Protocol Workshop Series – Interim Report: Workshop 1, Bethesda, Maryland, March 2000.

Short G. F., S. Y. Golovine, S. M. Hecht (1999): Effects of release factor 1 on *in vitro* protein translation and the elaboration of proteins containing unnatural amino acids. *Biochemistry* **38**, 8808-8819.

PUBLICATIONS AVAILABLE AT WORKSHOP

Bond, W. B., M. S. Favero (1970) Thermal profile of a *Bacillus* species (ATCC 27380) extremely resistant to dry heat. *Applied Microbiology* **29** (6): 859-860.

Bond, W. W., M. S. Favero, N. J. Petersen, J. H. Marshall (1970): Dry-heat inactivation kinetics of naturally occurring spare populations. *Applied and Environmental Microbiology* **20** (4): 573-578.

Bond, W. W., M. S. Favero, N. J. Petersen, J. H. Marshall (1971): Relative frequency distribution of D_{125C} values for spore isolates from the Mariner-Mars 1969 spacecraft. *Applied Microbiology* **21** (5): 832-836.

Brown, P., E. H. Rau, B. K. Johnson, A. E. Bacote, C. J. Gibbs, Jr., D. C. Gajdusek (2000): New studies on the heat resistance of hamster-adapted scrapie agent: Threshold survival after ashing at 600°C suggests an inorganic template of replication. *Proc. Natl. Acad. Sci. USA* **97** (7): 3418-3421.

Favero, M. S. (1971): Microbiologic assay of space hardware. *Environmental Biology and Medicine* **1**: 27-36.

Favero, M. S. (1978): Public health considerations associated with a Mars surface sample return mission. *COSPAR: Life Sciences and Space Research* **XVI**: 33-37.

Fisher, D. A., I. J. Pflug (1977): Effect of combined heat and radiation on microbial destruction. *Applied and Environmental Microbiology* **33** (5): 1170-1176.

Puleo, J. R., M. S. Favero G. S. Oxborrow, C. M. Herring (1975): Method for collecting naturally occurring airborne bacterial spores for determining their thermal resistance. *Applied Microbiology* **30** (5): 786-790.